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<b>(21) International Application Number:</b> PCT/US97/07726  <b>(22) International Filing Date:</b> 7 May 1997 (07.05.97)  <b>(30) Priority Data:</b> 60/017,427 8 May 1996 (08.05.96) US 60/019,300 7 June 1996 (07.06.96) US 60/021,859 16 July 1996 (16.07.96) US Not furnished 10 April 1997 (10.04.97) US  <b>(60) Parent Application or Grant</b> <b>(63) Related by Continuation</b> US 60/017,427 (CIP) Filed on 8 May 1996 (08.05.96)  <b>(71) Applicant (for all designated States except US):</b> BIOGEN, INC. [US/US]; 14 Cambridge Center, Cambridge, MA 02142 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> SANICOLA-NADEL, Michele [US/US]; 4 Maple Road, Winchester, MA 01890 (US). HESSION, Catherine [US/US]; 35 Otis Hill Road,		Hingham, MA 02043 (US). CATE, Richard, L. [US/US]; 64 Arrowhead Road, Weston, MA 02193 (US).  <b>(74) Agent:</b> LEVINE, Leslie, M.; Biogen, Inc., 14 Cambridge Center, Cambridge, MA 02142 (US).  <b>(81) Designated States:</b> <del>AL, AM, AT, AU, AZ, BA, BB, BG, BR,</del> <del>BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE,</del> <del>GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,</del> <del>LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ,</del> <del>PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT,</del> <del>UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW,</del> <del>SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD,</del> <del>RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES,</del> <del>FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent</del> <del>(BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD,</del> <del>TG).</del>  <b>Published</b> <i>Without international search report and to be republished          upon receipt of that report.</i>
<b>(54) Title:</b> RET LIGAND (RetL) FOR STIMULATING NEURAL AND RENAL GROWTH  <b>(57) Abstract</b>  This invention relates to nucleotide sequences which encode a Ret ligand (RetL9, as well as to methods of stimulating neural and renal growth by treating cells and mammalian subjects with RetL DNA or protein. The invention provides a purified and isolated DNA molecule coding for a RetL, having the nucleotide sequence of any RetL, but specifically including rat retL 1 cDNA (SEQ ID NO:1), partial human RetL1 cDNA (SEQ ID NO:8), full length human retL 1 cDNA (SEQ ID NO:10), human retL2 cDNA (SEQ ID NO:12), murine retL3 cDNA (SEQ ID NO:12) or human retL3 cDNA (SEQ ID NO:16), partial human retL3 cDNA (SEQ ID NO:18) or human retL3 cDNA (SEQ ID NO:20). The invention further provides a RetL protein, with an amino acid sequence comprising that of rat RetL1 (SEQ ID NO:2), partial human RetL1 (SEQ ID NO:9), full length human RetL (SEQ ID NO:11), human RetL2 (SEQ ID NO:13), murine RetL3 (SEQ ID NO:17), partial human RetL3 (SEQ ID NO:19) or human RetL3 (SEQ ID NO:21).		

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## RET LIGAND (RetL) FOR STIMULATING NEURAL AND RENAL GROWTH

**FIELD OF THE INVENTION**

This invention relates to nucleotide sequences which encode a Ret ligand (RetL), as well as to methods of stimulating neural and renal growth by treating cells and mammalian subjects with RetL DNA or protein.

**BACKGROUND OF THE INVENTION**

5 One of the goals of current research on cell signaling and receptor activation is to enable therapeutic modulation of processes involved in cell growth and survival. Such processes determine outcome in diverse medical conditions, including organ failure, fetal development, and tumor growth, among others. Each of these conditions is of worldwide clinical importance, and has limited efficacious treatment options. It is an object of the invention to provide compositions  
10 and methods for promoting regeneration or survival of damaged tissue, as well as for treating disorders involving the aberrant growth and development of tissues.

Tissue loss or end-stage organ failure affects millions of people worldwide each year and adds substantially to health care costs. Organ or tissue loss is usually treated by transplanting organs from donors, by surgical reconstruction, or with mechanical devices. Each of these  
15 remedies has shortcomings. Transplantation is limited by donor shortage, surgical reconstruction can create other long-term problems, and mechanical devices cannot perform all the functions of a single organ, and therefore cannot prevent progressive deterioration. Thus, a real medical need exists for new solutions to these problems.

Protein factors that affect the growth, differentiation and/or survival of cells may be  
20 useful in the treatment of disorders of organs which contain responsive cells. Factors or ligands that interact with receptors of the receptor protein tyrosine kinase (RPTK) family are of particular interest in this regard. These receptors are involved in many cellular programs including cell growth and differentiation, and the genesis of many neoplasias. Thus the factors or ligands that interact with these receptors may prove useful in treating disorders of certain

organs where the tissue has been damaged. Alternatively, it may be useful to block the interaction of these factors with their receptors in order to block tumor growth.

The Ret proto-oncogene encodes a receptor tyrosine kinase that is expressed during development in a variety of tissues, including the peripheral and central nervous systems and the kidney. The abnormalities present in ret null mice suggest that Ret is critical for the migration and innervation of enteric neurons to the hindgut, and for proliferation and branching of the ureteric bud epithelium during kidney development (Nature 367, 380-383, 1994). The search for a key component of the Ret signaling pathway, the Ret ligand, has been an area of intensive research.

#### SUMMARY OF THE INVENTION

The invention provides a purified and isolated DNA molecule coding for a RetL, having the nucleotide sequence of any RetL, but specifically including rat retL1 cDNA (SEQ ID NO:1), partial human retL1 cDNA (SEQ ID NO:8), full-length human retL1 cDNA (SEQ ID NO:10), human retL2 cDNA (SEQ ID NO:12), murine retL3 cDNA (SEQ ID NO:16), partial human retL3 cDNA (SEQ ID NO:18) or human retL3 cDNA (SEQ ID NO:20). The invention further provides a RetL protein, with an amino acid sequence comprising that of rat RetL1 (SEQ ID NO:2), partial human RetL1 (SEQ ID NO:9), full-length human RetL1 (SEQ ID NO:11), human RetL2 (SEQ ID NO:13), murine RetL3 (SEQ ID NO:17), partial human RetL3 (SEQ ID NO:19) or human RetL3 (SEQ ID NO:21).

In another embodiment, the invention includes a DNA sequence which encompasses the sequence (partial human retL1 cDNA (SEQ ID NO:8)) of the insert DNA of clone HRL20, which is ATCC No. 97604, or the sequence of the insert DNA of clone #230-5A-86-17 (rat retL1 cDNA (SEQ ID NO:1)), which is ATCC No. 98047.

In another embodiment of the invention, a purified and isolated DNA molecule for use in securing expression in a prokaryotic or eukaryotic host cell of a polypeptide product has at least a part of the primary structural conformation and the biological activity of RetL; the DNA may be a) a DNA molecule which comprises rat retL1 cDNA, partial human retL1 cDNA, full-length human retL1 cDNA, human retL2 cDNA, murine retL3 cDNA or human retL3 cDNA, or the complementary strand of rat retL1 cDNA, partial human retL1 cDNA, full-length human retL1 cDNA, human retL2 cDNA, murine retL3 cDNA or human retL3 cDNA; b) DNA molecules



which hybridize under stringent conditions to the DNA molecules defined in a) or fragments thereof; or c) DNA molecules which, but for the degeneracy of the genetic code, would hybridize to the DNA molecules defined in a) and b). A purified and isolated DNA molecule coding for a polypeptide fragment or variant of a human RetL having the biological activity of a RetL is also  
5 within the invention.

Any of the recombinant DNA molecules of the invention may be operably linked to an expression control sequence.

Also included within the invention are vectors and delivery systems which encompass the DNA molecules or constructs defined elsewhere in this specification. The vector may  
10 encompass a DNA molecule encoding a RetL or a variant of a RetL.

The invention includes prokaryotic or eukaryotic host cells stably transformed or transfected by a vector comprising a DNA molecule encoding a native or variant RetL.

A purified and isolated human RetL substantially free of other human proteins is specifically within the invention, as is a process for the production of a polypeptide product  
15 having part or all of the primary structural conformation and the biological activity of a RetL. Such a process may include the steps of growing, under suitable culture conditions, prokaryotic or eukaryotic host cells transformed or transfected with any DNA molecule of the invention, in a manner allowing expression of such polypeptide product, and recovering a RetL. The polypeptide product of the expression in a prokaryotic or eukaryotic host cell of a DNA is also  
20 included.

The invention also includes proteins and protein fragments, variants and derivatives, whether soluble or membrane bound. In selected embodiments, the protein has an amino acid sequence which comprises rat RetL1, partial human RetL1, full-length human RetL1, human RetL2, murine RetL3, or human RetL3, or is a variant of one of these sequences. In other  
25 embodiments, the protein is a fusion protein including Ret or a RetL, fused to another molecule or molecular fragment, such as an immunoglobulin, toxin, imageable compound or radionuclide. Also included are chimeric molecules of RetL.

Other embodiments of the invention include specific monoclonal antibodies to a RetL of the invention. Such an antibody may be associated with a toxin, imageable compound or  
30 radionuclide. The invention also includes hybridoma cell lines which produce specific antibodies to Ret, including AA.FF9, AA.HE3, AF.E9, BA.B1, BB.B6, AA.GE7, CD.F11,

AH.E3, CD.G4, AG.E7, BD.G6 and BH.G8, as well as subclones of these hybridomas, and the antibodies produced by these hybridomas or subclones of these hybridomas.

The invention further includes a method of promoting growth of new tissue, or promoting survival of damaged tissue in a subject, including administering to the subject a therapeutically effective amount of a compound which interacts with cellular Ret and thereby induces autophosphorylation of Ret. The compound may be RetL1, RetL2, or RetL3, a fragment of a full-length RetL, or an antibody which binds to Ret. The compound may be administered concurrently with a therapeutically effective amount of a second compound, such as GDNF, neurturin or a GDNF-related molecule. While tissues of interest for these methods may include any tissue, preferred tissues include renal tissue, neural tissue, heart, stomach, small intestine, spinal cord, or lung. In one embodiment, the RetL is a soluble RetL. The subject of the methods may be human.

In another method of the invention, Ret signal transduction between a first cell expressing a RetL and a second cell is inhibited by contacting the first cell with a soluble Ret protein or with an antibody to the RetL. The soluble Ret protein may be a fusion protein.

The invention also includes a method for targeting a toxin, imageable compound or radionuclide to a cell expressing Ret, encompassing contacting the cell with a RetL fusion protein or an anti-Ret antibody conjugated to a toxin, imageable compound or radionuclide. The RetL can be RetL1, RetL2 or RetL3. In another method, growth of a tumor cell which expresses Ret is suppressed, with a step of the method being contacting the cell with a fusion protein of a RetL and a toxin or radionuclide, or an anti-Ret antibody conjugated to a toxin or radionuclide. The cell may be within a subject, and the protein or the conjugated antibody is administered to the subject.

Also encompassed within the invention is a method for targeting a toxin, imageable compound or radionuclide to a cell expressing a RetL, comprising contacting the cell with a fusion protein comprising Ret and a toxin, imageable compound or radionuclide, or an anti-RetL antibody conjugated to a toxin, imageable compound or radionuclide. Another embodiment includes the method of suppressing growth of a tumor cell which expresses a RetL, comprising contacting the cell with a fusion protein of Ret and a toxin or radionuclide or with an anti-RetL antibody conjugated to a toxin or radionuclide; the cell may be within a subject, and the protein administered to the subject.

The RetL for any of the methods of the invention can be RetL1, RetL2 or RetL3, or a variant or fragment of RetL1, RetL2 or RetL3.

Methods of gene therapy are also within the invention. One embodiment is a method of treating a subject with a disorder of Ret metabolism, comprising administering to the subject a vector comprising a DNA molecule encoding a RetL, as well as a method of promoting growth of new tissue in a subject, comprising administering such a vector to the subject. Another embodiment includes a method of promoting survival of damaged tissue in a subject, one step of the method being administering a therapeutically effective amount of a vector encoding a RetL to the subject.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a cDNA sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of rat RetL1. The nucleotide sequence extends from base pair 201 through base pair 1700 of SEQ ID NO:1, and contains the entire open reading frame.

FIGURE 2A is a partial cDNA sequence (SEQ ID NO:8) and deduced amino acid sequence (SEQ ID NO:9) of human RetL1. This sequence is that of the insert of clone HRL20, deposited as ATCC No. 97604.

FIGURE 2B is a composite full-length DNA sequence (SEQ ID NO:10) and deduced amino acid sequence (SEQ ID NO:11) of human RetL1.

FIGURE 3A is a comparison of the nucleotide sequence of human RetL1 (upper line of sequence) with that of rat RetL1 sequence (lower line of sequence). Vertical lines between nucleotides show identity at a position, while a dot indicates a gap at that position.

FIGURE 3B is a comparison of the amino acid sequence of human RetL1 (upper line of sequence) with that of rat RetL1 sequence (lower line of sequence). Vertical lines between corresponding amino acids show identity at a residue, while a dot indicates a conservative substitution at that residue.

FIGURE 4A is a schematic diagram of a possible role for Ret and RetL in the interaction between a metanephric mesenchyme cell and a ureteric bud cell.

FIGURE 4B is a schematic diagram of a method of screening transfectants of a cDNA library for clones that express a RetL. The presence of expressed RetL on transfectants is detected by  
5 assessing the binding by those transfectants either of Ret/IgG fusion protein or of Ret/alkaline phosphatase fusion protein.

FIGURE 5 is a schematic diagram showing the construction of the plasmids used to express the rat Ret/IgG fusion protein.

FIGURE 6 is a schematic diagram showing the construction of the plasmids used to express the  
10 human Ret/IgG fusion protein.

FIGURE 7 is a cDNA sequence (SEQ ID NO:12) and deduced amino acid sequence (SEQ ID NO:13) of human retL2, as found in clone DSW240. The protein reading frame is contained within nucleotides 25 to 1416.

FIGURE 8 is a comparison of the amino acid sequence of human RetL2 (upper line of sequence)  
15 with that of human RetL1 sequence (lower line of sequence). Vertical lines between amino acids show identity at a position, while a dot indicates a gap at that position.

FIGURE 9 is a cDNA sequence (SEQ ID NO:16) and deduced amino acid sequence (SEQ ID NO:17) of murine RetL3.

FIGURE 10 is a cDNA sequence (SEQ ID NO:20) and deduced amino acid sequence (SEQ ID  
20 NO:21) of human RetL3.

## **DETAILED DESCRIPTION OF THE INVENTION**

### **Sequence Identification Numbers**

Nucleotide and amino acid sequences referred to in the specification have been given the following sequence identification numbers:

- 5        SEQ ID NO:1 - rat retL1 cDNA
- SEQ ID NO:2 - rat RetL1 aa
- SEQ ID NO:3 - oligomer kid-13
- SEQ ID NO:4 - oligomer kid-14
- SEQ ID NO:5 - oligomer kid-15
- 10        SEQ ID NO:6 - extracellular rat retL1 cDNA
- SEQ ID NO:7 - extracellular rat RetL1 aa
- SEQ ID NO:8 - partial human retL1 cDNA
- SEQ ID NO:9 - partial human RetL1 aa
- SEQ ID NO:10 - human retL1 cDNA
- 15        SEQ ID NO:11 - human RetL1 aa
- SEQ ID NO:12 - human retL2 cDNA
- SEQ ID NO:13 - human RetL2 aa
- SEQ ID NO:14 - partial murine retL3 cDNA (EST AA50083)
- SEQ ID NO:15 - partial murine RetL3 aa
- 20        SEQ ID NO:16 - murine retL3 cDNA
- SEQ ID NO:17 - murine RetL3 aa
- SEQ ID NO:18 - partial human retL3 cDNA
- SEQ ID NO:19 - partial human RetL3 aa
- SEQ ID NO:20 - human retL3 cDNA
- 25        SEQ ID NO:21 - human retL3 aa

### **Definitions**

As used herein, the term "RetL" means any protein which specifically interacts with the receptor protein Ret, and which when it interacts with Ret triggers Ret dimerization and/or autophosphorylation of the tyrosine kinase domain of Ret. The DNA sequences which code for

RetL and for Ret are termed "retL" and "ret", respectively. A ligand may be soluble, or present as a membrane-bound molecule on the same or on a different cell as the Ret molecule for which it is triggering autophosphorylation. In certain uses or interactions with Ret, the ligand may require additional molecules to trigger autophosphorylation. Ligands of the invention include co-receptors or accessory ligand cofactors. Ligands of the invention further include anti-Ret mAbs which act as Ret antagonists, triggering Ret dimerization and autophosphorylation. The ligand may also be modified in various ways, such as incorporated as a portion of a fusion protein, such as with a toxin or radionuclide.

By "alignment of sequences" is meant the positioning of one sequence, either nucleotide or amino acid, with that of another, to allow a comparison of the sequence of relevant portions of one with that of the other. An example of one method of this procedure is given in Needleman et al. (J. Mol. Biol. 48:443-453, 1970). The method may be implemented conveniently by computer programs such as the Align program (DNASTAR, Inc.). As will be understood by those skilled in the art, homologous or functionally equivalent sequences include functionally equivalent arrangements of the cysteine residues within the conserved cysteine skeleton, including amino acid insertions or deletions which alter the linear arrangement of these cysteines, but do not materially impair their relationship in the folded structure of the protein. Therefore, internal gaps and amino acid insertions in the candidate sequence are ignored for purposes of calculating the level of amino acid sequence homology or identity between the candidate and reference sequences. One characteristic frequently used in establishing the homology of proteins is the similarity of the number and location of the cysteine residues between one protein and another.

By "cloning" is meant the use of in vitro recombination techniques to insert a particular gene or other DNA sequence into a vector molecule. In order to successfully clone a desired gene, it is necessary to employ methods for generating DNA fragments, for joining the fragments to vector molecules, for introducing the composite DNA molecule into a host cell in which it can replicate, and for selecting the clone having the target gene from amongst the recipient host cells.

By "cDNA" is meant complementary or copy DNA produced from an RNA template by the action of RNA-dependent DNA polymerase (reverse transcriptase). Thus a "cDNA clone" means a duplex DNA sequence complementary to an RNA molecule of interest, carried in a cloning vector.

By "cDNA library" is meant a collection of recombinant DNA molecules containing cDNA inserts which together comprise a representation of the mRNA molecules present in an entire organism or tissue, depending on the source of the RNA templates. Such a cDNA library may be prepared by methods known to those of skill, and described, for example, in Maniatis et al., Molecular Cloning: A Laboratory Manual, *supra*. Generally, RNA is first isolated from the cells of an organism from whose genome it is desired to clone a particular gene. Preferred for the purposes of the present invention are mammalian, and particularly human, cell lines. Alternatively, RNA may be isolated from a tumor cell, derived from an animal tumor, and preferably from a human tumor. Thus, a library may be prepared from, for example, a human adrenal tumor, but any tumor may be used.

As used herein, the term "DNA polymorphism" refers to the condition in which two or more different nucleotide sequences can exist at a particular site in DNA.

"Expression vector" includes vectors which are capable of expressing DNA sequences contained therein, i.e., the coding sequences are operably linked to other sequences capable of effecting their expression. It is implied, although not always explicitly stated, that these expression vectors must be replicable in the host organisms either as episomes or as an integral part of the chromosomal DNA. A useful, but not a necessary, element of an effective expression vector is a marker encoding sequence, which is a sequence encoding a protein which results in a phenotypic property (e.g. tetracycline resistance) of the cells containing the protein which permits those cells to be readily identified. In sum, "expression vector" is given a functional definition, and any DNA sequence which is capable of effecting expression of a specified contained DNA code is included in this term, as it is applied to the specified sequence. Such vectors are frequently in the form of plasmids, so "plasmid" and "expression vector" are often used interchangeably. However, the invention is intended to include such other forms of expression vectors, including phage, which serve equivalent functions and which may from time to time become known in the art.

Similarly, a "functional derivative" of a gene of any of the proteins of the present invention is meant to include "fragments", "variants", and "analogues" of the gene, which may be "substantially similar" in nucleotide sequence, and which encode a molecule possessing similar activity.

"GDNF-related molecule" means any molecule which is at least 40% homologous to either GDNF or neurturin, and is also capable of specifically binding a RetL.

The term "gene" means a polynucleotide sequence encoding a peptide.

By "homogeneous" is meant, when referring to a peptide or DNA sequence, that the  
5 primary molecular structure (i.e., the sequence of amino acids or nucleotides) of substantially all molecules present in the composition under consideration is identical.

The term "oligonucleotide" as used herein in referring to probes, oligomer fragments to be detected, oligomer controls, unlabeled blocking oligomers and primers for amplification of sequences is defined as a molecule comprised of more than three deoxyribonucleotides or  
10 ribonucleotides. Its exact size will depend on many factors, which in turn depend on the ultimate function or use of the oligonucleotide.

The term "probe" refers to a ligand of known qualities capable of selectively binding to a target antiligand. As applied to nucleic acids, the term "probe" refers to a strand of nucleic acid having a base sequence complementary to a target strand.

15 "Recombinant host cells" refers to cells which have been transformed with vectors constructed using recombinant DNA techniques. As defined herein, the antibody or modification thereof produced by a recombinant host cell is by virtue of this transformation, rather than in such lesser amounts, or more commonly, in such less than detectable amounts, as would be produced by the untransformed host.

20 As used herein, the terms "restriction endonucleases" and "restriction enzymes" refer to bacterial enzymes each of which cut double-stranded DNA at or near a specific nucleotide sequence.

As used herein, the term "restriction fragment length polymorphism" ("RFLP") refers to the differences among individuals in the lengths of a particular restriction fragment.

25 A molecule is said to be "substantially similar" to another molecule if the sequence of amino acids in both molecules is substantially the same, and if both molecules possess a similar biological activity. Thus, provided that two molecules possess a similar activity, they are considered variants as that term is used herein even if one of the molecules contains additional amino acid residues not found in the other, or if the sequence of amino acid residues is not  
30 identical. As used herein, a molecule is said to be a "chemical derivative" of another molecule when it contains additional chemical moieties not normally a part of the molecule. Such



moieties may improve the molecule's solubility, absorption, biological half life, etc. The moieties may alternatively decrease the toxicity of the molecule, eliminate or attenuate any undesirable side effect of the molecule, etc. Moieties capable of mediating such effects are disclosed, for example, in Remington's Pharmaceutical Sciences, 16th ed., Mack Publishing Co., Easton, Penn. (1980).

By "vector" is meant a DNA molecule, derived from a plasmid or bacteriophage, into which fragments of DNA may be inserted or cloned. A vector will contain one or more unique restriction sites, and may be capable of autonomous replication in a defined host or vehicle organism such that the cloned sequence is reproducible.

By "substantially pure" is meant any protein of the present invention, or any gene encoding any such protein, which is essentially free of other proteins or genes, respectively, or of other contaminants with which it might normally be found in nature, and as such exists in a form not found in nature.

### **Compounds of the Invention**

The invention includes cDNA coding for a RetL, such as the nucleotide sequence of rat retL1 cDNA, partial human retL1 cDNA, full-length human retL1 cDNA, human retL2 cDNA, murine retL3 cDNA or human retL3 cDNA. In addition, the compounds of the invention include sequences which include the above sequences, or are derivatives of one of these sequences. The invention also includes vectors, liposomes and other carrier vehicles which encompass one of these sequences or a derivative of one of these sequences. The invention also includes proteins transcribed and translated from rat retL1 cDNA, partial human retL1 cDNA, full-length human retL1 cDNA, human retL2 cDNA, murine retL3 cDNA or human retL3 cDNA, including but not limited to rat RetL1, partial human RetL1, full-length human RetL1, human RetL2, murine RetL3, or human RetL3, and their derivatives and variants.

One embodiment of the invention includes soluble variants of a RetL. Soluble variants lack at least a portion of the intramembrane section of the native RetL. In some examples, the soluble variant lacks the phosphatidylinositol glycan linkage of the native RetL. Soluble variants include fusion proteins which encompass derivatives of RetL that lack a phosphatidylinositol motif.

5 Variants can differ from naturally occurring RetL in amino acid sequence or in ways that do not involve sequence, or both. Variants in amino acid sequence are produced when one or more amino acids in naturally occurring RetL is substituted with a different natural amino acid, an amino acid derivative or non-native amino acid. Particularly preferred variants include naturally occurring RetL, or biologically active fragments of naturally occurring RetL, whose sequences differ from the wild type sequence by one or more conservative amino acid substitutions, which typically have minimal influence on the secondary structure and hydrophobic nature of the protein or peptide. Variants may also have sequences which differ by one or more non-conservative amino acid substitutions, deletions or insertions which do not abolish the RetL biological activity. Conservative substitutions typically include the substitution of one amino acid for another with similar characteristics such as substitutions within the following groups: valine, glycine; glycine, alanine; valine, isoleucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. The non-polar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

20 Other conservative substitutions can be taken from the table below, and yet others are described by Dayhoff in the Atlas of Protein Sequence and Structure (1988).

TABLE 1: CONSERVATIVE AMINO ACID REPLACEMENTS

For Amino Acid	Code	Replace with any of
Alanine	A	D-Ala, Gly, beta-Ala, L-Cys, D-Cys
Arginine	R	D-Arg, Lys, homo-Arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Asparagine	N	D-Asn, Asp, D-Asp, Glu, D-Glu, Gln, D-Gln
Aspartic Acid	D	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	C	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr
Glutamine	Q	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	E	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	G	Ala, D-Ala, Pro, D-Pro, Beta-Ala, Acp
Isoleucine	I	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	L	D-Leu, Val, D-Val, Met, D-Met
Lysine	K	D-Lys, Arg, D-Arg, homo-Arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Methionine	M	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val, Norleu
Phenylalanine	F	D-Phe, Tyr, D-Thr, L-Dopa, His, D-His, Trp, D-Trp, Trans 3,4 or 5-phenylproline, cis 3,4 or 5 phenylproline
Proline	P	D-Pro, L-I-thioazolidine-4-carboxylic acid, D- or L-1-oxazolidine-4-carboxylic acid

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Serine	S	D-Ser, Thr, D-Thr, allo-Thr, Met, D-Met, Met(O), D-Met(O), Val, D-Val
Threonine	T	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Met(O), D-Met(O), Val, D-Val
Tyrosine	Y	D-Tyr, Phe, D-Phe, L-Dopa, His, D-His
Valine	V	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met

Other variants within the invention are those with modifications which increase peptide stability. Such variants may contain, for example, one or more non-peptide bonds (which replace the peptide bonds) in the peptide sequence. Also included are: variants that include residues other than naturally occurring L-amino acids, such as D-amino acids or non-naturally occurring or synthetic amino acids such as beta or gamma amino acids and cyclic variants. Incorporation of D- instead of L-amino acids into the polypeptide may increase its resistance to proteases. See, e.g., U.S. Patent 5,219,990.

The peptides of this invention may also be modified by various changes such as insertions, deletions and substitutions, either conservative or nonconservative where such changes might provide for certain advantages in their use. Splice variants are specifically included in the invention.

In addition to substantially full-length polypeptides, the present invention provides for biologically active fragments of the polypeptides. A RetL polypeptide or fragment is biologically active if it exhibits a biological activity of naturally occurring RetL. Such biological activities include the ability to specifically bind the extracellular portion of Ret, with an affinity that is at least 50% of, and preferably at least equal to, the affinity of naturally occurring RetL for the extracellular portion of Ret. Another biological activity is the ability to bind to an antibody which is directed at an epitope which is present on naturally occurring RetL.

In other embodiments, variants with amino acid substitutions which are less conservative may also result in desired derivatives, e.g., by causing changes in charge, conformation and other biological properties. Such substitutions would include for example, substitution of hydrophilic residue for a hydrophobic residue, substitution of a cysteine or proline for another residue, substitution of a residue having a small side chain for a residue having a bulky side chain or substitution of a residue having a net positive charge for a residue having a net negative charge. When the result of a given substitution cannot be predicted with certainty, the derivatives may be readily assayed according to the methods disclosed herein to determine the presence or absence of the desired characteristics.

Generally, substitutions that may be expected to induce changes in the functional properties of Ret polypeptides are those in which: (i) a hydrophilic residue, e.g., serine or threonine, is substituted by a hydrophobic residue, e.g., leucine, isoleucine, phenylalanine, or alanine; (ii) a cysteine residue is substituted for (or by) any other residue; (iii) a residue having

an electropositive side chain, e.g., lysine, arginine or histidine, is substituted for (or by) a residue having an electronegative charge, e.g., glutamic acid or aspartic acid; or (iv) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having such a side chain, e.g., glycine.

5 Variants within the scope of the invention include proteins and peptides with amino acid sequences having at least sixty percent homology with rat RetL1 (SEQ ID NO:2), partial human RetL1 (SEQ ID NO:9), full-length human RetL1 (SEQ ID NO:11), human RetL2 (SEQ ID NO:13), murine RetL3 (SEQ ID NO:17), partial human RetL3 (SEQ ID NO:19) or human RetL3 (SEQ ID NO:21). More preferably the sequence homology is at least eighty, at least  
10 ninety percent, or at least ninety-five percent. For the purposes of determining homology the length of comparison sequences will generally be at least 8 amino acid residues, usually at least 20 amino acid residues. Variants of the compounds of the invention also includes any protein which 1) has an amino acid sequence which is at least forty percent homologous to a RetL protein of the invention, and also which 2) after being placed in an optimal alignment with the  
15 RetL sequence (as depicted for RetL1 and RetL2 in Figure 8), has at least 80% of its cysteine residues aligned with cysteines in the RetL protein of the invention.

Just as it is possible to replace substituents of the scaffold, it is also possible to substitute functional groups which are bound to the scaffold with groups characterized by similar features. Such modifications do not alter primary sequence. These will initially be conservative, i.e., the  
20 replacement group will have approximately the same size, shape, hydrophobicity and charge as the original group. Non-sequence modifications may include, for example, in vivo or in vitro chemical derivatization of portions of naturally occurring RetL, as well as changes in acetylation, methylation, phosphorylation, carboxylation or glycosylation.

Also included within the invention are agents which specifically bind to a protein of the  
25 invention, or a fragment of such a protein. These agents include Ig fusion proteins and antibodies (including single chain, double chain, Fab fragments, and others, whether native, humanized, primatized, or chimeric). Additional descriptions of these categories of agents are in PCT application 95/16709, the specification of which is herein incorporated by reference.

## **EXPERIMENTAL PROCEDURE**

### **Overview of Strategy**

The general strategy used to clone RetL1 is shown in Figures 4A and 4B. Our strategy was based on the premise that at least a RetL is expressed on the metanephric mesenchyme of the developing kidney as a membrane protein (although it is possible that the ligand is also expressed in a soluble form; Figure 4A). The RetL interacts with the Ret receptor on the ureteric bud cell, activating its tyrosine kinase cytoplasmic domain and sending a signal to the nucleus, which in turn activates genes involved in the growth and branching of the ureteric bud. Therefore, proteins containing the extracellular domain of Ret fused to either the F<sub>c</sub> portion of human immunoglobulin G1 (IgG1) or alkaline phosphatase (AP) can be used as part of a strategy to clone RetL as shown in Figure 4B. The fusion proteins, the expression libraries and other reagents used in the cloning of RetL1 are described below.

We first isolate a cDNA for rat RetL1 and then use it as a probe to isolate a cDNA for human RetL1. cDNAs are subsequently isolated for RetL2 and RetL3.

### **Generation of Reagents Required for Direct Expression Cloning of Ret Ligands**

#### **1. Isolation of cDNA Encoding Rat Ret Extracellular Domain**

To identify RetL1, fusion proteins are generated consisting of the extracellular domains of either rat or human Ret fused to a protein, in one example the human Fc portion of IgG1, and in another example alkaline phosphatase. Both fusion partners can be easily assayed to detect cells which express the ligand as illustrated in Figure 4B.

Since a cDNA coding for rat Ret has never been disclosed, we isolate a cDNA encoding the extracellular domain of the rat Ret receptor using the Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) method. We compare the two nucleotide sequences for human (Genbank Accession numbers M57464 and X15262) and murine (Genbank Accession number X67812) ret and design oligonucleotide primers from regions of high identity between the two sequences. A sense oligomer called kid-013 (SEQ ID NO:3; contains nucleotides 150-169 of Genbank sequence X15262) is chosen from the 5' end of the human ret cDNA sequence overlapping the ATG initiation codon. It includes nucleotides on its 5' end encoding a Not I restriction site for the purpose of cloning. Two antisense oligomers called kid-014 (SEQ ID

NO:4; contains the complement of nucleotides 1819-1839 of Genbank sequence M57464) and kid-015 (SEQ ID NO:5; contains the complement of nucleotides 1894-1914 of Genbank sequence X67812) are chosen, respectively, from the human and murine cDNA sequences immediately 5' to the sequences that encode the transmembrane domains. Oligomers kid-014 and kid-015 contain additional nucleotides at their 5' ends that encode a SalI restriction site for the purpose of cloning.

Total RNA is isolated from the day 14 embryonic rat kidney and mRNA is purified using oligo-dT chromatography. mRNA is converted to cDNA using AMV reverse transcriptase and the cDNA is converted to double stranded cDNA and amplified using Taq polymerase in a standard polymerase chain reaction with oligomers kid-013 and kid-015. The synthesis of a 1942 bp PCR fragment is confirmed by running an aliquot of the PCR reaction on a 1% agarose gel. The rest of the PCR fragment is digested with NotI and SalI and cloned into pSAB132 previously digested with NotI and SalI. The resulting plasmid is called pJC011. The entire insert of plasmid pJC011 contained between the NotI and SalI sites is sequenced, and is shown as extracellular rat retL1 cDNA, SEQ ID NO:6. A translation of this sequence reveals the peptide sequence (SEQ ID NO:7) for extracellular rat RetL1. Because oligomers for PCR were chosen from human and mouse sequences of ret, it is possible that the nucleotide sequence shown as that of extracellular rat ret cDNA, and the peptide sequence shown as that of extracellular rat Ret, may differ from the natural rat ret nucleotide and Ret peptide sequences in the regions of kid-013 and kid-015 sequences. Subsequently, ret cDNA clones are isolated from a day 18 rat embryonic kidney cDNA library and a few nucleotide changes are observed in the primer regions resulting in two amino acid changes. One change is in the signal sequence (arginine at position 5 to threonine) and one change is near the end of the extracellular domain (glutamic acid at position 633 to alanine). Both of these changes should not affect ligand binding.

## 2. Ret/IgG Fusion Proteins

Fusion proteins are generated consisting of the extracellular domains of the rat (aa residues #1-637) and human (aa residues #1-636) Ret receptors fused to the Fc portion of human IgG1.

The construction of the plasmids used to express the rat Ret/IgG fusion protein is shown schematically in Figure 5. In order to construct a gene encoding the rat Ret/IgG fusion protein,



we digest pJC011 (described above ) containing the rat Ret extracellular domain with SalI, and ligate it to a 700 bp SalI fragment from plasmid 2-4, to create plasmid pJC012. This SalI fragment contains part of the Fc domain of human IgG1 originally derived from plasmid pSAB144. Plasmid 2-4 was created previously via a three way ligation: a NotI - SalI fragment generated by PCR containing the extracellular domain of the rabbit TGF-beta type II receptor; a 693 bp SalI - NotI fragment from pSAB144 containing part of the Fc domain of human IgG1; and NotI digested pSAB132. As shown in Figure 5, a fragment containing the Fc domain can be released from the 2-4 plasmid as a 700 bp SalI fragment. pJC012 is transfected into COS cells and the rat Ret/IgG fusion protein is purified from the medium 48 hrs later using Protein-A Sepharose chromatography. In order to make a stable cell line producing the rat Ret/IgG protein, the 2612 bp NotI fragment from pJC012 containing the entire rat Ret/IgG fusion protein is isolated and cloned into the NotI site of expression vector pMDR901. The resulting plasmid is called pJC022. Plasmid pJC022 is transfected into CHO cells to generate stable cell lines. The highest producing cell line is suspension adapted. Typical yields for the rat Ret/IgG CHO line are 75mg/L.

The construction of the plasmids used to express the human Ret/IgG fusion protein is shown schematically in Figure 6. In order to construct a gene encoding the human Ret/IgG fusion protein, we obtain a plasmid containing a cDNA encoding the human Ret receptor from Dr. M.Takahashi (Department of Pathology, Nagoya University, School of Medicine, Nagoya, Japan). A PCR fragment is generated from this plasmid using oligomers kid-013 and kid-014. The PCR fragment is treated with Klenow fragment followed by digestion with NotI to produce a PCR fragment with a sticky NotI end and one blunt end. This fragment is cloned into the vector pGEM11zf(+) previously digested with EcoRI, treated with Klenow fragment, and digested with NotI, in order to generate a sticky NotI end and one blunt end. The resulting plasmid is called pJC013. The 1916 bp NotI - SalI fragment from pJC013 is isolated after a complete digestion with NotI and a partial digestion with SalI, and ligated to the 693 bp SalI - NotI fragment from pSAB144 containing part of the Fc domain of human IgG1, and the pSAB132 expression vector digested with NotI. The resulting plasmid is called pJC015. The insert in plasmid pJC013 is sequenced and found to contain a single nucleotide difference which changes one amino acid in the extracellular domain of human Ret (Genbank sequence M57464 has a C at position 812, whereas pJC013 has a T at the corresponding position; this results in a

change in amino acids from alanine to valine at position 294 of the human Ret protein sequence). This nucleotide is corrected back to the C residue specified by Genbank sequence M57464 by site specific mutagenesis of plasmid pJC013, producing plasmid pJC023. A 585 bp BstE2 fragment from pJC023 containing the repaired nucleotide sequence is isolated and cloned into plasmid pJC015 from which the 585 bp BstE2 fragment containing the variant nucleotide has been removed. The new plasmid is called pJC024. The 2609 bp NotI fragment from pJC024 containing the entire human Ret/IgG fusion protein is isolated and cloned into the NotI site of expression vector pMDR901. The resulting plasmid is called pJC025. Plasmid pJC025 is transfected into CHO cells to generate stable cell lines. The highest producing cell line is suspension adapted. Typical yields for the human Ret/IgG CHO line are 6 mg/L.

Further details on production of the vectors employed in the methods of the invention are given in PCT applications 94/01456 and 92/02050, the specifications of which are herein incorporated by reference.

### 3. Bioactivity of the Ret/IgG Fusion Proteins

To determine if the Ret/IgG fusion proteins that we produce are bioactive and therefore would be good screening reagents for the cloning of a RetL, we perform several organ culture assays for bioactivity. The organ culture assay consists of growing day 13-14 embryonic rat kidneys in organ culture for 3-5 days in the presence of the Ret/Ig fusion protein at a concentration of 50 ug/ml. Kidneys are also cultured in the presence of LFA-3TIP/IgG or vehicle buffer. After the culture period, some of the kidneys are stained with the fluorescent lectin Dolichos Biflorus Agglutinin (DB lectin) which stains the collecting duct tissues, which are epithelial cells derived from the ureteric bud. These "DB" positive cells mark the Ret-positive cells, since Ret is expressed in the ureteric bud and its epithelial derivatives. This provides a gross assessment of the Ret/IgG fusion protein on the growth and development of the embryonic kidney. There is a clear difference in collecting duct morphology and growth between kidneys which have been cultured with LFA-3TIP and those cultured with the rat Ret/IgG fusion protein.. The Ret/IgG-treated kidneys have collecting ducts which show significantly less branching and are typically smaller overall.

Paraffin sections are prepared from other kidneys for histological examination.

Embryonic kidneys are treated with control buffer or with Ret/IgG, then stained with

hematoxylin and eosin. The Ret/Ig-treated embryonic kidney exhibits less branching of the collecting ducts than the control buffer-treated embryonic kidneys. In addition, Ret/IgG-treated kidneys have fewer tubules. We have also observed this effect with the human Ret/IgG fusion protein. These observations are consistent with the fusion proteins blocking the inductive signal  
5 between the mesenchyme and the ureteric bud. Therefore we conclude that the fusion protein is a good reagent for cloning a RetL.

#### 4. Ret/alkaline phosphatase Fusion Protein

Receptor/alkaline phosphatase (AP) fusion proteins have been used successfully to identify and clone ligands for c-kit (Cell 63:185, 1990), ligands for members of the eph family of  
10 orphan receptors (Cell 79:157, 1994), and recently to clone a receptor for leptin, the product of the ob gene (Cell 83:1263, 1995). Plasmids encoding the rat Ret/AP fusion protein are constructed and the rat Ret/AP protein is produced in COS7 cells in cell factories. Subsequently, a stable NIH3T3 cell line is generated expressing on average 10 mg/L of fusion protein. SDS-PAGE analysis of the rat Ret/AP protein indicates that its size is consistent with the predicted  
15 molecular weight, and gel filtration analysis indicates that it is produced as a dimer. Partial purification is achieved by affinity chromatography on an anti-AP column.

#### 5. Anti-Ret Antibodies

A rabbit polyclonal antibody is generated against the rat Ret/IgG fusion protein. The antibody works on Western blots, FACS analysis of Ret positive cell lines, and  
20 immunohistochemistry of embryonic kidney sections.

A panel of hamster anti-rat Ret monoclonal antibodies is generated. Rat Ret/IgG fusion protein, coupled to Protein A Sepharose, is used to immunize Armenian hamsters. 316 clones are obtained after the fusion and screened for their ability to bind rat Ret fusion proteins and/or human IgG in an ELISA assay. 11 clones produce antibodies that bind only to rat Ret/IgG (and  
25 rat Ret/AP), but not human IgG. The cross reactivity to human Ret is assayed by FACS; four clones produce antibodies that can bind to the Ret positive human cell line THP-1. The following table summarizes the Ret binding properties of twelve monoclonal antibodies.

Clone	ELISA rat Ret/Ig	FACS human THP-1
AA.FF9.5	+	-
AA.HE3.7	+	+
AF.E9.5	+	-
BA.B1.16	+	-
BB.B6	+	-
AA.GE7.3	+	-
CD.F11.2	+	-
AH.E3.11	+	+
CD.G4.2	+	+
AG.E7.9	+	-
BD.G6	+	+
BH.G8	-	-

#### 6. cDNA Expression Libraries

We prepare cDNA libraries from rat embryonic kidney, one in the CDM8 vector which utilizes the SV40 origin for amplification, and one in a modified InVitrogen vector, pCEP4, which utilizes the EBV origin for amplification. This modified vector, CH269, has the EBNA-1 gene sequence removed. The EBNA-1 protein interacts with the EBV origin, but the gene is not needed on the vector when cells are used which stably express the EBNA protein. The library in the CDM8 vector contains  $1.5 \times 10^6$  clones with an average insert size of 1.18 kb, while the library in the CH269 vector contains approximately  $1 \times 10^6$  clones with an average insert size of 1.5 kb.

#### Expression Cloning of Ret Ligand RetL1

##### A. Cloning of Rat Ret Ligand RetL1

##### 1. Initial Attempts at Expression Cloning of Ret Ligand RetL1 -

A number of direct expression methods have been tried to clone RetL1. All of these methods are based on the concept illustrated in Figure 4B. cDNAs from a cDNA library are introduced into mammalian cells; cells that receive RetL1 can be identified using the Ret fusion proteins. Although the three approaches described below were unsuccessful, important  
5 knowledge and expertise was acquired, which was deployed in a subsequent approach that met with success.

a. Panning Method with Ret/IgG - The rat Ret/IgG fusion protein is used in an attempt to isolate RetL1 by direct expression cloning using a panning method (Aruffo and Seed, Proc. Natl. Acad. Sci. 84: 8753-8757 (1987)). A day 18 embryonic rat kidney cDNA library in CDM8 is  
10 used for the panning effort. Pools of cDNAs from this library (5,000 - 10,000 cDNAs per pool) are introduced into COS cells using the DEAE-dextran method. After 48 hours, the cells are removed from the plates with EDTA, incubated with the fusion protein, and subsequently  
panned on plates coated with anti-human IgG, antibody. DNA is recovered from cells that adhered, transformed back into E. coli, and subsequently isolated for a second round of panning.  
15 We are unable to see any cells bind after the third round of panning, and very few clones are obtained after transformation of the Hirt DNA back into E. Coli. A VCAM cDNA, used in conjunction with an anti-VCAM monoclonal antibody as a positive control, could only be diluted to a ratio of 1:100 and still be detected, indicating that our pool sizes are probably too large. Analysis of some of the clones that are obtained after the second round of panning,  
20 indicates that the clones are undergoing rearrangement and deletion.

b. Preparative FACS Method with Ret/IgG - 80,000 cDNA clones from the day 18 embryonic rat kidney library (CDM8 vector) are introduced into COS7 cells and subjected to preparative FACS using the rat Ret/IgG protein followed by a fluor-tagged secondary antibody. The top 0.5% and 0.9% of fluorescing cells are collected and the plasmid DNA is recovered by  
25 Hirt lysis. The DNA is electroporated back into E. coli: 228 clones are obtained for the 0.5% pool and 752 clones for the 0.9% pool. DNA is recovered from the bacterial clones and a second round of preparative FACS is performed. Plasmids recovered from bacterial clones at the end of the second round are analyzed and found to contain large deletions and rearrangements.

c. Colorimetric Detection Method with Ret/AP - COS cells are transfected with 400 pools of the cDNA clones (1000 clones per pool) from the day 18 rat embryonic kidney cDNA library (CDM8 vector) and stained with the Ret/AP protein and a colorimetric substrate for alkaline phosphatase. The transfected cells are inspected under a microscope for positive signals. In one experiment, five potential positives were re-analyzed, but all were negative.

As a control for the Ret/AP protein, a VCAM/AP protein is produced by fusing the first two domains of human VCAM to the N-terminus of placental AP. (VCAM binds to the integrin VLA4, which is composed of two chains, alpha-4 and beta-1). Transient transfections of COS cells produces sufficient VCAM/AP protein for control experiments. The VCAM/AP protein is compared to VCAM/IgG directly coupled to AP, and to VCAM/IgG plus an AP coupled secondary antibody, in order to assess their ability to detect VLA4 on COS cells transfected with the alpha-4 chain cDNA (COS cells already express the beta-1 chain). The results show that while the VCAM/AP protein could detect VLA4 on transfected cells, the best detection is afforded by the VCAM/IgG protein in combination with an AP coupled secondary antibody.

d. Methodological Conclusions:

Three major conclusions emerged from these initial cloning efforts:

1) Methods which require that plasmid DNA be recovered for subsequent rounds (i.e. panning and preparative FACS) are not suitable when the abundance of the target cDNA is low, because of rearrangements and deletions that occur during these subsequent rounds. Based on the low expression of Ret, there is good reason to suspect that the expression of RetL1 is also low. The preferred approach is to transfect in pools and use a detection method that allows a positive pool to be identified. The original pool can then be broken down, with no need to recover the transiently expressed DNA from transfected cells.

2) The Ret/IgG protein when coupled to a secondary reagent affords better detection capability than the Ret/AP protein.

3) Control experiments with a VCAM/IgG control protein (and an AP coupled secondary antibody) and the alpha-4 integrin cDNA (diluted into CDM8 vector and transfected into COS cells) indicate that our detection capability is just about one in a thousand (i.e. the pool size cannot exceed 1000 clones). To attain an improved level of sensitivity, we changed from an SV40 origin based vector (expressed in COS cells) to an EBV origin based vector (expressed in

EBNA positive cell lines). EBV origin based vectors are maintained as episomes and are not as toxic to the cell as the SV40 origin based vectors after amplification. Considerable evidence exists that genes can be expressed at higher levels in these vectors and that cDNAs can be diluted much further (i.e. up to 1 to 80,000) and still be detected.

## 2. Screening of Pools from the EBV Origin Based cDNA library

We screen pools of clones from the day 18 rat embryonic kidney cDNA library (CH269 vector with the EBV origin) with the rat Ret/IgG fusion protein. In one experiment, 256 pools, each containing 5000 clones from the library, are generated. Briefly, an aliquot of the cDNA library is titrated, 5000 cells are plated (256 times), and are allowed to grow overnight. The colonies are scraped into medium: part of the culture is used to generate a glycerol stock for the pool (stored at -70) and part is used for a plasmid preparation. DNAs from the 256 pools are individually transfected into 293/EBNA cells ( $8 \times 10^5$  on a 60 mm plate) using the lipofection method. After 48 hr. the cells are washed two times with HBHA buffer (0.5 mg/ml BSA, 0.1%  $\text{NaN}_3$ , 20 mM HEPES (pH 7.0)) and incubated with 20 ug/ml rat Ret/IgG in Tris-buffered saline plus 1 mM  $\text{MgCl}_2$  and  $\text{CaCl}_2$  for 60-90 min at RT. Following this incubation, the cells are washed four times with HBHA buffer and then fixed with 60% acetone/3% formaldehyde/20 mM HEPES (pH 7.0) for 30 sec. Following two washes with HBS buffer (150 mM  $\text{NaCl}$ , 20 mM HEPES (pH 7.0)), the cells are incubated with an AP-coupled secondary antibody (goat anti-human IgG Fc-gamma-specific F(ab')<sub>2</sub>, (Jackson Immuno Research Laboratories; catalog # 109-056-098; 1:5000 dilution in Tris-buffered saline plus 1 mM  $\text{MgCl}_2$  and  $\text{CaCl}_2$ ) for 60 min at RT. The cells are then washed twice with HBS buffer and twice with AP substrate buffer (100 mM Tris-HCl (pH 9.5), 100 mM  $\text{NaCl}$ , 5 mM  $\text{MgCl}_2$ ) containing 2X Pierce Immuno Pure<sup>R</sup> Phosphatase suppressor (catalog #35002). The last wash is left for 15 min. The AP substrates NBT (0.33 mg/ml) and BCIP (0.17 mg/ml) are then added in AP substrate buffer containing the Pierce AP inhibitor and incubated with the cells for 5-20 min. The plates are then washed twice with water. The plates are then inspected under a dissecting microscope for the presence of purple stained cells.

From an analysis of the 256 pools, 17 positive pools are identified in the primary screen. DNA from each positive pool is re-transfected into 293/EBNA cells and the above procedure repeated along with some additional control experiments to confirm that the staining observed is

Ret/IgG specific. 10 out of the 17 positive pools only show staining with Ret/IgG fusion protein and not with another IgG fusion protein.

### 3. Breakdown of Pool #230

As an example, one of the above-described positive pools, designated #230, is broken  
5 down into smaller subpools in order to identify the cDNA within the pool that is conferring  
binding to the Ret/IgG fusion protein. 600 cells from the glycerol stock for pool #230 are plated  
(10 times) and grown overnight. Colonies on these plates are scraped into medium: one tenth of  
the culture is used to generate a glycerol stock and the remaining portion is used for a DNA  
preparation. The ten subpools of 600 clones are designated 230-1A through 230-5A and 230-1B  
10 through 230-5B. DNAs from these subpools are transfected into 293/EBNA cells and the  
procedure described above for staining with the Ret/IgG fusion protein is repeated. One subpool  
#230-5A is positive for staining with the Ret/IgG protein.

Pool #230-5A is further broken down in order to identify the cDNA with this subpool  
that is conferring binding to the Ret/IgG fusion protein. Cells from the glycerol stock of pool  
15 230-5A are plated and grown overnight. Colonies are picked into the wells of seven 96-well  
Bioblocks® and grown overnight. From each 96-well Bioblock, 4 pools of 20 clones and 1 pool  
of 16 clones are made. Thus 35 pools are generated from the seven Bioblocks® designated 230-  
5A-71 through 230-5A-105. DNAs are prepared from each of these pools and transfected into  
293/EBNA cells and re-assayed with the Ret/IgG fusion protein as described above. Pool #230-  
20 5A-86 is positive.

Pool #230-5A-86 is broken down by going back to the Bioblock and identifying the 20  
clones that were mixed together to make this pool. DNAs are made from all twenty clones and  
transfected individually into 293/EBNA cells and re-assayed for Ret/IgG as described above.  
Pool #230-5A-86-17 is found to be positive.

### 25 4. Characterization of Clone #230-5A-86-17

Clone #230-5A-86-17 (called retL-17 or clone 17 and deposited as ATCC 98047) is  
further analyzed by DNA sequencing. The entire nucleotide sequence of the insert of this clone  
is SEQ ID NO:1 (rat retL1 cDNA), and part of the nucleotide sequence is shown in Figure 1.

Within this nucleotide sequence, we find a reading frame coding for a protein of 468 amino acids



(rat RetL1). The predicted protein has a signal sequence with a predicted cleavage after amino acid 24 (Von Heijne et al., Nucl. Acid Res. 14:14683 (1986)). The hydrophobic C-terminus indicates that the protein may be linked to the cell via a phosphatidylinositol glycan linkage. There are three predicted N-linked glycosylation sites. These properties are consistent with those expected for a ligand for Ret.

We can express soluble forms of the rat RetL1 protein by truncating the gene prior to the hydrophobic C terminus. For example, this could be done by truncating after Lysine 435 (rat RetL1). Truncation upstream of this amino acid should also result in the expression of a soluble form of the rat RetL1 protein. The soluble rat RetL1 protein can be expressed by itself or as a part of a fusion with human immunoglobulin, a histidine tag, or a small epitope that is recognized by an antibody.

#### **B. Cloning of Human Ret Ligand RetL1**

A human embryonic kidney cDNA library in the vector lambda gt10 is purchased from Clontech (catalog #HL5004A). One million plaque forming units from the phage stock are plated on 10 Nunc™ plates. Duplicate plaque lifts are made on Schleicher and Schuell Optitran™ filters.

A probe is generated by digesting plasmid rat RetL1 with the restriction enzyme PvuII, followed by agarose gel isolation of a 1.34 kb fragment which corresponds to nt 242-1582 of the rat RetL nucleotide sequence (rat retL1 cDNA). This coding region probe is P<sup>32</sup> labeled by random priming (Feinberg and Vogelstein, Anal. Biochem. 137:266-267, 1984). The filters are hybridized overnight in 300 ml plaque screen PSB buffer (50 mM Tris pH 7.5, 1M NaCl, 0.1% sodium pyrophosphate, 0.2% PVP, and 0.2% Ficoll) containing 10% dextran sulphate, 100 ug/ml tRNA, and 6.7 X 10<sup>7</sup> CPM of the rat probe, at 55C. They are washed twice with plaque screen buffer and twice with 2XSSC/1%SDS at 55C and exposed to film at -70C with an intensifying screen.

Duplicate positives are cored from the master plates into SM (100 mM NaCl, 10 mM SO<sub>4</sub>, 50 mM Tris pH 7.5) plus gelatin. 24 of these positives are plaque purified. Lambda miniprep DNA from the purified candidate plaques is digested with NotI, electrophoresed on 1% agarose gel and Southern blotted. The Southern blot is hybridized with the rat RetL1 coding region probe. Clone HRL20 has the longest insert (4.4 kb) which hybridizes intensely to the rat probe. DNA sequence (partial human retL1 cDNA; SEQ ID NO: 8; Figure 2A) and deduced

peptide sequence (partial human RetL1; SEQ ID NO: 9; Figure 2A) have been obtained from this clone, confirming that it is the human homologue. This clone encodes most of the coding region, including the 3' end of the coding region.

To obtain the 5' end of the human cDNA, a human fetal kidney Marathon-Ready™ cDNA kit is purchased from Clontech (catalog #7423-1). Antisense oligonucleotides Kid-155, corresponding to the complement of nucleotides 62-81 of SEQ ID NO: 8 (partial human retL1 cDNA) and Kid-154, corresponding to the complement of nucleotides 17-43 of SEQ ID NO: 8 (partial human retL1 cDNA) are synthesized. PCR is performed using the Advantage™ cDNA PCR kit (Clontech catalog #8417-1) combined with Marathon™ cDNA reagents and the oligonucleotides Kid-155 or Kid-154. The first PCR reaction is set up as follows: 35.5 ul H<sub>2</sub>O; 5.0 ul 10X KlenTaq Buffer; 1.0 ul 10mM dNTP mix; 1.0 ul 50X Advantage™ KlenTaq Polymerase mix. These reagents are combined and mixed. Then 5.0 ul Marathon-Ready™ Fetal Kidney cDNA; 1.0 ul 10uM AP1 primer and 1.5 ul 6.4uM Kid-155 are added (final volume = 50 ul). PCR is carried out in a Perkin-Elmer Cetus DNA Thermal Cycler 480 with the following cycle conditions: 1 cycle of 94C for 1 min; 30 cycles of 94C for 30 sec, 55C for 30 sec, 68C for 4 min. A nested PCR is performed using the product of the first PCR reaction. First, 5 ul of PCR product #1 is diluted 50 fold with TE (final volume 250 ul). The nested PCR reaction contains 35.5 ul H<sub>2</sub>O; 5.0 ul 10X KlenTaq Buffer; 1.0 ul 10mM dNTP mix; 1.0 ul 50X Advantage™ KlenTaq Polymerase mix. These reagents are mixed as above. 5.0 ul diluted PCR product #1: 1.0 ul 10uM AP2 primer and 1.5 ul 6.9uM Kid-154 are then added. Cycle conditions are the same as above. The resultant product of approximately 700 bp is purified on a 1% low-melt agarose gel and phenol extracted. The purified DNA is cloned into the EcoR5 site of pZerO™ (Invitrogen catalog #K2510-01). Sequence information is obtained from multiple isolates, including clones called HRL7G6 and HRL7G8.

The sequence obtained from clone HRL7G8 is found to overlap with the sequence of clone HRL20 (partial human retL1 cDNA) and is used to generate a full-length sequence of human RetL1 (full-length human retL1 cDNA), also shown in Figure 2B. The nucleotide sequence obtained from clone HRL7G8 represents nucleotides 1 to 502 of full-length human retL1 cDNA; the nucleotide sequence from clone HRL20 represents nucleotides 460 to 1682 of full-length human retL1 cDNA. The sequence from clone HRL7G8 is confirmed by sequencing another cDNA clone (GJ102) isolated from the human embryonic kidney lambda gt10 cDNA

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library described above, using a probe derived from clone HRL7G6. Nucleotides 118 to 1497 comprise the protein reading frame of full-length human retL1 cDNA.

The complete amino acid sequence of human RetL1 is also shown in Figure 2B. As shown by the BESTFIT analysis depicted in Figure 3A, the human retL1 cDNA is 88.2% identical to the rat retL1 cDNA. The peptide comparison (Figure 3B) shows the human putative peptide sequence to be 93.3% identical, and 97.2% similar, to that of the rat.

### Cloning of Ret Ligand RetL2

#### **A. Cloning of Human RetL2**

The peptide sequence of rat RetL1 (rat RetL1) is used to search the GenBank database with the program BLAST in order to identify related proteins (i.e. isoforms). BLAST, or Basic Local Alignment Search Tool, uses the method of Altschul et al. (J. Mol. Biol. 215: 403-410, 1990) to search for similarities between a query sequence and all the sequences in the sequence database. The query sequence and the database to be searched can be either peptide or nucleotide in any combination. When the rat RetL1 peptide sequence is queried against the Expressed Sequence Tag (EST) nucleotide database, two significant matches are obtained. One is with GenBank Accession # R02249, a 229 bp EST from a combined human fetal liver and spleen cDNA library, and the other is with Genbank Accession # H12981, a 521 bp EST from a human infant brain cDNA library. The two ESTs share 99% identity in a region of overlap indicating that they are from the same cDNA. Oligonucleotides are generated from the H12981 EST: KID-228 (GAA TGA CAA CTG CAA GAA GCT GCG CTC CTC; corresponding to nucleotides 38-67 and also to nucleotides 534-563 of SEQ ID NO:12), and antisense oligonucleotide KID-229 (GTG TAC TCG CTG GGC ACC CG; corresponding to the complement of nucleotides 156-175 and also to the complement of nucleotides 652-671 of SEQ ID NO:12).

1X10<sup>6</sup> plaque forming units from a Clontech Human Fetal Liver 5' -Stretch Plus lambda GT10 cDNA library (cat# HL5003a) are screened in duplicate on OPTITRAN<sup>TM</sup> filters. The filters are hybridized with <sup>32</sup>P-labeled oligonucleotides KID-228 and KID-229 in 400 mls plaque screen buffer (50mM Tris pH 7.5, 1M NaCl, 0.1% sodium pyrophosphate, 0.2% Polyvinylpyrrolidone and 0.2% Ficoll) containing 10% Dextran sulfate and 100ug/ml tRNA and

80 pmole each  $^{32}\text{P}$ -labeled oligonucleotide at 65C overnight. They are washed twice with 2X SSC/1% SDS and twice with 1X SSC/1% SDS and exposed to film. 11 duplicate positives are purified. DNA from each of these clones is analyzed by restriction enzyme digest followed by agarose gel electrophoresis and Southern blotting. The filters are hybridized to KID-228 and KID-229 to confirm that the inserts hybridize to the probe. The insert of clone DSW240 is completely sequenced (human retL2 cDNA, SEQ ID NO:12) and is shown in Figure 7.

Nucleotides 25-1416 comprise the protein reading frame of human retL2 cDNA, which encodes a protein of 464 amino acids (human RetL2; SEQ ID NO:13), and is shown in Figure 7. As shown by the BESTFIT analysis depicted in Figure 8, the human RetL2 protein is 49.1% identical and 63.7% similar to the human RetL1 protein. It shares in common with human RetL1 a hydrophobic N-terminus indicative of a signal sequence and a hydrophobic C-terminus indicative of a phosphatidylinositol glycan linkage motif. In addition, 30 cysteines out of the 31 that are present in each protein are conserved.

#### B. Demonstration that RetL2 is a Ligand for Ret

We demonstrate that RetL2 is a ligand for Ret by transfecting 293/EBNA cells with an expression plasmid that contains the insert of clone DSW240 and by showing that the cells can bind a soluble Ret/IgG fusion protein.

The insert of DSW240 is removed using NotI and cloned into the expression vector CH269 which contains an EBV origin and allows for high expression in EBNA positive cell lines. Restriction digests are performed to identify clones that have the correct orientation. Plasmid DNA is prepared from a clone having the correct orientation.

Plasmid DNAs (the retL2 expression plasmid, a retL1 expression plasmid for a positive control, and an expression plasmid containing an unrelated protein for a negative control) are transfected into 293/EBNA cells ( $8 \times 10^5$  on a 60 mm plate) using the lipofection method.

After 48 hr, the cells are washed two times with HBHA buffer (0.5 mg/ml BSA, 0.1%  $\text{NaN}_3$ , 20 mM HEPES (pH 7.0)) and incubated with 20 ug/ml rat Ret/IgG in Tris-buffered saline plus 1 mM  $\text{MgCl}_2$  and  $\text{CaCl}_2$  for 60-90 min at room temperature. Following this incubation, the cells are washed four times with HBHA buffer and then fixed with 60% acetone/3% formaldehyde/20 mM HEPES (pH 7.0) for 30 sec. Following two washes with HBS buffer (150 mM NaCl, 20 mM HEPES (pH 7.0)), the cells are incubated with an AP-coupled secondary antibody (goat

anti-human IgG Fc-gamma-specific F(ab'), (Jackson Immuno Research Laboratories: catalog # 109-056-098; 1:5000 dilution in Tris-buffered saline plus 1 mM MgCl<sub>2</sub> and CaCl<sub>2</sub>) for 60 min at RT. The cells are then washed twice with HBS buffer and twice with AP substrate buffer (100 mM Tris-HCl (pH 9.5), 100 mM NaCl, 5 mM MgCl<sub>2</sub>) containing 2X Pierce Immuno

5 Pure®Phosphatase suppressor (catalog #35002). The last wash is left for 15 min. The AP substrates NBT (0.33 mg/ml) and BCIP (0.17 mg/ml) are then added in AP substrate buffer containing the Pierce AP inhibitor and incubated with the cells for 5-20 min. The plates are then washed twice with water. The plates are then inspected under a dissecting microscope for the presence of purple stained cells. The presence of purple stained cells indicates that the

10 Ret/fusion protein has bound to the cells and that the RetL2 protein is a ligand for Ret. Purple stained cells are also observed after trasfection with the retL1 expression vector but not with the negative control vector.

### Cloning of Ret Ligand RetL3

#### **A. Murine RetL3**

15 A search of the EST data base with rat RetL1 amino acid sequence discloses two murine ESTs with homology to Ret ligands. These ESTs are AA049894, and AA050083 (which is partial murine retL3 cDNA, SEQ ID NO:14). Plasmids encoding these ESTs are obtained from Genome Systems Inc. (Catalog# 475791 and #475497) as bacterial stabs. Plasmid DNA is prepared from single colonies obtained by streaking the stabs onto LB Amp plates. The inserts

20 from these plasmids are sequenced in their entirety. Comparison of the two sequences demonstrates that AA049894, which has a 1.4 kb insert, is contained within AA050083, which has a 1.9 kb insert. Translation of the DNA sequence from AA050083 indicates there is a continuous open reading frame from NT205 to NT1242 (partial murine RetL3; SEQ ID NO:15). This ORF had 37.5% identity to that of rat retL1 and 40.2% identity to rat retL2. However, the

25 open reading frame does not encode a Met or a signal sequence at the 5' end. We examine the 5' ORFs upstream of this region and find a Met in the context of a Kozak consensus sequence for translation iniation and a potential signal sequence for surface expression/secretion. This ORF is out of frame with the downstream ORF indicating that EST AA050083 contains a potential mutation, such as an insertion, deletion, intron or cloning artifact, at its 5' end.

In order to obtain the correct 5' end, we employ Marathon RACE. Mouse 11-day embryo Marathon-Ready™ cDNA (cat.#7458-1) and an Advantage™ Kit (cat.# 8417-1) is purchased from Clontech. Antisense oligonucleotides, Kid-366, corresponding to the complement of nucleotides 847-866 of SEQ ID NO:14 and Kid-365, corresponding to the complement of nucleotides 589-615 of SEQ ID NO:14 are synthesized. PCR is performed using and an Advantage™ cDNA PCR kit (Clontech cat.#8417-1) combined with Marathon™ cDNA reagents and oligonucleotide Kid-366. The first PCR reaction is set up as follows: 35.3 ul H<sub>2</sub>O; 5.0 ul 10X KlenTaq Buffer; 1.0ul 10mM dNTP mix; 1.0 ul 50X Advantage™ KlenTaq Polymerase mix. These reagents are combined and mixed. Then 5.0 ul Marathon-Ready™ mouse 11-day embryo cDNA; 1.0 ul 10uM AP1 primer and 1.7ul 5.88uM Kid-366 are added (final volume = 50ul). PCR is carried out in a Perkin-Elmer Cetus DNA Thermal Cycler 480 with the following cycle conditions: 1 cycle of 94C for 1 min; 5 cycles of 94C for 30 sec, 72C for 4 min; 5 cycles of 94C for 30 sec, 70C for 4 min; 25 cycles 94C for 30 sec, 68C for 4 min. A nested PCR is performed using the product of the first PCR reaction. First, 5 ul of PCR product#1 is diluted 50 fold with TE( Final volume 250 ul). The nested PCR reaction contains 35.5 ul H<sub>2</sub>O; 5.0 ul 10X KlenTaq Buffer; 1.0 ul 10mM dNTP mix; 1.0 ul 50X Advantage™ KlenTaq Polymerase mix. These reagents are mixed as above. 5.0 ul diluted PCR product #1; 1.0 ul 10uM AP2 primer and 3.6 ul 2.8uM Kid-365 are then added. Cycle conditions are the same as above. The resultant product of approximately 665 bp is purified on a 1% low-melt agarose gel and Qiaex II™ (Qiagen cat#20021) extracted. The purified DNA is cloned into pNoTA/T7™ using PRIME PCR CLONER™ cloning system (5 Prime-> 3 Prime cat.#1-725029). Sequence information is obtained from multiple isolates, including clones called DSW252 and DSW253.

The sequence of DSW252 is found to overlap with SEQ ID NO:14 except that an additional T is present between NT 252 and NT 253 of the SEQ ID NO:14 sequence. This T is also present in the other isolates DSW251 and DSW253. Insertion of this additional base corrects the ORF such that a single 1191 bp ORF (counting from the first Met) encoding 397 amino acids is obtained. This ORF encodes a Met in the context of a canonical translation initiation consensus sequence (Kozak) and includes a signal sequence for surface expression/secretion.

To obtain a full-length murine clone which is capable of being expressed, a 630 bp NotI-BamHI fragment of DSW252 and a 1308 bp BamHI-NotI fragment of AA050083 is purified

and ligated to Not 1 digested expression vector CH269. The ligation is transformed into E.coli XL1-Blue (Stratagene cat. #200236). Qiawell Ultra minipreps are performed on resultant transformants. These are analysed by restriction digest and gel electrophoresis for correct size and for orientation. This construct is called DSW254. The insert of DSW254 is sequenced in its entirety (murine retL3; SEQ ID NO:16) and the ORF is confirmed as encoding a protein of 397 amino acids (murine RetL3; SEQ ID NO:17). These sequences are also shown in Figure 9. The C-terminus of RetL3 is hydrophobic and indicative of a phosphatidylinositol glycan linkage motif.

### B. Human RetL3

In order to find a candidate tissue source for cloning human RetL3, we utilize northern blots of mouse tissues to determine the expression pattern of murine RetL3. Of the tissues surveyed, expression of RetL3 is highest in heart tissue. A human adult heart cDNA library in the vector lambda gt10 is purchased from Clontech (catalog# HL3026a). One million plaque forming units from the phage stock are plated on 10 Nunc plates. Duplicate plaque lifts are made on Schleicher and Schuell Optitran™ filters.

A probe is generated by PCR with primers Kid-366 and Kid-367, corresponding to nucleotides 397-420 of the AA050083 sequence. PCR reaction is set up as follows: 10 ul 10X PFU Buffer, 2.0 ul 10mM dNTP mix, 72.1 ul H<sub>2</sub>O, 3.1 ul 13.2 uM Kid-367, 6.8 ul 5.88 uM Kid-366, 5.0 ul 0.1 ug/ ul AA050083 DNA and 2.0 ul 2.5 Units/ul PFU (Stratagene catalog # 600154) are mixed. PCR is carried out in Perkin-Elmer Cetus DNA Thermal Cyclor 480 with the following conditions: 25 cycles of 94 C for 1 min, 53C for 1 min., 72C for 4 min. The product is purified by extraction with phenol, chloroform, isoamyl alcohol 50:49:1 followed by low-melt agarose gel electrophoresis and QiaexII purification of the excised fragment. This coding region probe is P<sup>32</sup> labeled by random priming (Feinberg and Vogelstein). The filters are hybridized overnight in 200 ml Plaque screen buffer ( ) containing 10% dextran sulphate, 100 ug/ml tRNA and 1.8 X 10<sup>8</sup> CPM of the mouse probe at 65C. They are washed twice with plaque screen buffer, twice with 2XSSC/1% SDS, twice with 1XSSC/1%SDS at 65C and exposed to film at -70C with an intensifying screen. Duplicate positives are plaque purified. Lambda miniprep DNA from the purified candidate plaques is digested with EcoR1, electrophoresed on a 1% agarose gel and Southern blotted. The Southern blot is hybridized with the mouse probe. Clone GJ128 ,

which has a 1.3 kb insert, hybridizes intensely to the mouse coding region probe. DNA sequence (partial human retL3 cDNA; SEQ ID NO:18) and deduced peptide sequence (partial human RetL3; SEQ ID NO:19) are obtained from this clone, confirming that it is the human homologue. This clone encodes most of the coding region, including the 3' end of the coding region.

5 The 1.3 kb insert from GJ128 is purified, labeled with P<sup>32</sup> and used to screen the Clontech human adult heart library in order to obtain a clone with the 5' end. No clones containing the 5' end are obtained in a screen of 2 X 10<sup>6</sup> plaques from this library. Northern analysis of human adult tissue mRNA blots (Clontech catalog # 7760-1, 7759-1 and 7767-1) hybridized with the same probe, using protocols supplied by manufacturer, indicates that human RetL3 is expressed  
10 in human adult spinal cord, stomach, heart, pancreas, small intestine, colon, prostate and testis. A Clontech human adult spinal cord cDNA library (catalog # 5001a) is screened with GJ128 insert. 3 independent clones are purified and the longest, GJ135 is sequenced. The sequence of the insert of GJ135 overlaps with the insert of GJ128, allowing the generation of a composite sequence of the full-length human retL3 cDNA (SEQ ID NO:20) and the determination of the  
15 full-length human RetL3 (SEQ ID NO:21). These sequences are also shown in Figure 10. Human RetL3 is 34.3% and 34.9% identical to human RetL1 and human RetL2, respectively. It has 76.8% identity with murine RetL3.

### THERAPEUTIC USES OF THE COMPOUNDS OF THE INVENTION

Native and variant RetL's, anti-RetL antibodies, anti-Ret antibodies, and fusion proteins  
20 of Ret and of RetL's may have therapeutic utility in situations where it is desirable to block or to activate the Ret signaling pathway, to stimulate renal and/or neuronal cell growth or survival in disease situations where these cells are lost or damaged, or to suppress growth of or to kill undesirable cells such as tumor cells that express Ret or a RetL.

In general, compounds of the invention that bind to Ret, inducing dimerization and/or  
25 autophosphorylation of Ret, are useful for stimulating growth of or limiting damage to Ret-expressing tissues. The compounds of the invention are useful for stimulating renal tissue growth and/or survival, supporting renal function, and in minimizing damage to renal tissue after various insults. Particular conditions which may be beneficially treated with the compounds of the invention include acute renal failure, acute nephritis, chronic renal failure, nephrotic  
30 syndrome, renal tubule defects, kidney transplants, toxic injury, hypoxic injury, and trauma.



Renal tubule defects include those of either hereditary or acquired nature, such as polycystic renal disease, medullary cystic disease, and medullary sponge kidney. This list is not limited, and may include many other renal disorders (see, e.g., Harrison's Principles of Internal Medicine, 13th ed., 1994, which is herein incorporated by reference.)

5 In other applications, the genes and proteins of the invention may be used to treat conditions where neural growth and regeneration is desirable. This would include any conditions involving disorders of neural degeneration, such as Alzheimer's disease, Parkinson's, Huntington's, Tourette's, amyotrophic lateral sclerosis, as well as motor neuron disease, demyelinating diseases such as multiple sclerosis, bacterial diseases such as meningitis, abscess,  
10 or empyema, viral diseases such as HIV-associated myelopathy, prion diseases including Creutzfeldt-Jakob disease. Also included are disorders of damage to neural tissue, whether caused by neoplastic impingement, trauma, or cerebrovascular events such as hemorrhage or emboli. Diseases of the cranial nerves and of the spinal cord, including disorders involving traumatic, inflammatory, congenital or vascular etiologies, are specifically included, as are  
15 disorders affecting the autonomic nervous system. Also included are developmental neural disorders such as mental retardation, autism, fetal alcohol syndrome, Down's syndrome, and cerebral palsy. The compounds of the invention may also be used to treat syndromes involving the peripheral nervous system. These disorders include those caused by any of the factors previously listed, and specifically include Lyme disease, HIV-associated neuropathies,  
20 polymyositis, muscular dystrophy, and myasthenia gravis.

Anti-RetL antibodies and Ret fusion proteins of the invention, which specifically bind to the protein of rat RetL, partial human RetL1, full-length human RetL1, human RetL2, murine RetL3 or human RetL3, or fragments of these proteins, are useful in several methods. The compounds may be used therapeutically to inhibit or block Ret receptor signaling, such as for  
25 blocking growth of tumors which depend on activation of Ret signaling for growth. These agents may also be fused to detectable markers, such as fluoroscopically or radiographically opaque substances, and administered to a subject to allow imaging of tissues which express a RetL. The agents may also be bound to substances, such as horseradish peroxidase, which can be used as immunocytochemical stains to allow visualization of areas of RetL-positive cells on  
30 histological sections. A specific antibody could be used alone in this manner, and sites where it is bound can be visualized in a sandwich assay using an anti-immunoglobulin antibody which is

itself bound to a detectable marker. Specific antibodies to any RetL are also useful in immunoassays to quantify the substance for which a given antibody has specificity. Specific antibodies to a RetL may also be bound to solid supports, such as beads or dishes, and used to remove the ligand from a solution, either for use in purifying the protein or in clearing it from the solution. Each of these techniques is routine to those of skill in the immunological arts.

Other methods of the invention include modulating Ret-RetL signaling by contacting Ret with an anti-Ret monoclonal antibody. The effect of such a mAb-Ret contact can be to either block or to stimulate activation of the Ret signaling pathway, depending on the characteristics of the interaction of each particular mAb with Ret. Certain mAbs interact with Ret as agonists, with the agonist mAb-Ret binding triggering the dimerization and autophosphorylation of Ret. Other mAbs act as Ret antagonists. The interaction of Ret with an antagonist mAb prevents Ret signaling activation by other RetL's, or by complexes comprising RetL's, which would otherwise activate the Ret signaling pathway.

A RetL and/or antibodies to Ret or to a Ret fusion protein can be used to allow imaging of tissues which express Ret, or in the immunohistological or preparative methods described above for antibodies to a RetL.

Fusion proteins encompassing a RetL and/or anti-Ret antibodies can be used to specifically target medical therapies against cancers and tumors which express Ret. Such tumors might include the several different tumor phenotypes which have been associated with mutations in Ret (N. Engl. J. Med. 335:943-951, 1996; Nature 367: 319-320, 1996; Trends Gen. 12:138-144, 1996). Therapeutic interventions against neoplasias which express a RetL utilize fusion proteins which incorporate Ret and/or an anti-RetL antibody. The anti-Ret antibody or anti-RetL antibody may be effective by itself through antibody-dependent and complement-dependent cytotoxicity mediated by the Fc domain. Such hybrid ligands and antibodies can be made more effective as cancer therapeutics by using them as delivery vehicles for antineoplastic drugs, toxins and cytotoxic radionuclides, such as yttrium 90. Cytotoxic effector cells may be targeted to tumor cells using heteroconjugate antibodies, where an antibody specific for either Ret or for a RetL expressed by a tumor is covalently coupled to an antibody directed against a surface protein on cytotoxic effector cells, such as NK cells or CTLs.

One example of an anti-Ret antibody or RetL therapy is to conjugate the toxic A chain of ricin or a modified full-length form of ricin (which can no longer bind cells) to a RetL or to an

antibody directed against the Ret polypeptide expressed on the surface of malignant cells. In another embodiment, a toxin is conjugated to Ret or to an anti-RetL antibody to selectively target and kill RetL-positive cells, such as a tumor expressing a RetL. Such an approach has proved successful with blocked ricin conjugated to a monoclonal antibody against the CD19 antigen expressed on most neoplastic cells (Grossbard et al., Blood 79:576,1992). Other toxins are equally useful, as known to those of skill in the art. Such toxins include, but are not limited to, pseudomonas exotoxin, diphtheria toxin, and saporin. This approach should prove even more successful using a RetL or anti-Ret antibody, as contrasted to the known anti-CD19 antigen approach, because Ret is expressed in a very limited number of tissues.

The above approaches, using fusions of ricin or other toxins, are equally applicable to toxic conjugates of RetL or of an anti-Ret antibody; these are useful for selectively targeting and killing Ret-positive cells, such as tumor cells expressing Ret.

Another approach to such medical therapies is to use radioisotope labeled RetL or anti-Ret antibodies. Such radiolabeled compounds will preferentially target radioactivity to tumor sites in cells expressing Ret, sparing normal tissues. Depending on the radioisotope employed, the radiation emitted from a radiolabeled antibody bound to a tumor cell may also kill nearby malignant tumor cells that do not express Ret. A variety of radionuclides may be used. Isotopes that emit  $\beta$  particles (for example,  $^{131}\text{I}$ ) have been successful when employed with monoclonal antibodies against CD20 present on B-cell lymphomas (Kaminski et al., N. Engl. J. Med. 329: 459 (1993); Press et al., N. Engl. J. Med. 329: 1219 (1993)). Radionuclides emitting  $\beta$  particles generate radioactive emissions that are tumoricidal over distances spanning several cell diameters, permitting the eradication of antigen negative cells and diminishing the consequences of nonhomogenous deposition of antibody or ligand in tumors.

Radionuclides emitting  $\alpha$  particles may also be employed. The low dose rate irradiation generated by radionuclide labeled RetL or anti-Ret antibodies may be more therapeutically effective than the instantaneous irradiation delivered externally in conventional radiation therapy. Low dose rate irradiation can induce apoptosis (programmed cell death) in certain cell lines (Macklis et al., Radiat. Res. 130: 220 (1992); Maklis et al., Radiopharm. 5: 339 (1992)).

The compounds of the invention are administered in therapeutically-effective amounts, which means an amount of a compound which produces a medically desirable result or exerts an influence on the particular condition being treated.

The term "subject" used herein is taken to mean any mammal to which Ret ligand or gene may be administered. Subjects specifically intended for treatment with the method of the invention include humans, as well as nonhuman primates, sheep, horses, cattle, goats, pigs, dogs, cats, rabbits, guinea pigs, hamsters, gerbils, rats and mice, as well as the organs, tumors, and cells derived or originating from these hosts.

### **Use of Compounds of the Invention in Gene Therapy**

The RetL genes of the invention are introduced into damaged tissue to stimulate production of a RetL by the transfected cells, to promote cell growth and/or survival of cells that express Ret.

In a specific embodiment of a gene therapy method a RetL gene may be introduced into a renal or neural target tissue of choice. A RetL would then be stably expressed and stimulate Ret receptor-positive cells to grow, divide, differentiate, and/or potentiate cell survival. Furthermore, RetL genes may be introduced into a target cell using a variety of well-known methods that use either viral or non-viral based strategies.

Non-viral methods include electroporation, membrane fusion with liposomes, high velocity bombardment with DNA-coated microprojectiles, incubation with calcium-phosphate-DNA precipitate, DEAE-dextran mediated transfection, and direct micro-injection into single cells. For instance, a RetL gene may be introduced into a cell by calcium phosphate coprecipitation (Pillicer et al., Science, 209: 1414-1422 (1980); mechanical microinjection and/or particle acceleration (Anderson et al., Proc. Natl. Acad. Sci. USA, 77: 5399-5403 (1980); liposome based DNA transfer (e.g., LIPOFECTIN-mediated transfection- Fefgner et al., Proc. Nat. Acad. Sci., USA, 84: 471-477, 1987; Gao and Huang, Biochim. Biophys. Res. Comm., 179: 280-285, 1991; DEAE Dextran-mediated transfection; electroporation (U.S. Patent 4.956.288); or polylysine-based methods in which DNA is conjugated to deliver DNA preferentially to liver hepatocytes (Wolff et al., Science, 247: 465-468, 1990; Curiel et al., Human Gene Therapy 3: 147-154, 1992).

Target cells may be transfected with the genes of the invention by direct gene transfer. See, e.g., Wolff et al., "Direct Gene Transfer Into Moose Muscle In Vivo", Science 247:1465-68, 1990. In many cases, vector-mediated transfection will be desirable. Any of the methods known in the art for the insertion of polynucleotide sequences into a vector may be used. See,

for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989) and Ausubel et al., *Current Protocols in Molecular Biology*, J. Wiley & Sons, NY (1992), both of which are incorporated herein by reference.

Promoter activation may be tissue specific or inducible by a metabolic product or administered substance. Such promoters/enhancers include, but are not limited to, the native RetL promoter, the cytomegalovirus immediate-early promoter/enhancer (Karasuyama et al., *J. Exp. Med.*, 169: 13 (1989)); the human beta-actin promoter (Gunning et al., *Proc. Nat. Acad. Sci. USA*, 84: 4831 (1987)); the glucocorticoid-inducible promoter present in the mouse mammary tumor virus long terminal repeat (MMTV LTR) (Klessig et al., *Mol. Cell. Biol.*, 4: 1354 (1984)); the long terminal repeat sequences of Moloney murine leukemia virus (MuLV LTR) (Weiss et al., *RNA Tumor Viruses*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1985)); the SV40 early region promoter (Bernoist and Chambon, *Nature*, 290:304 (1981)); the promoter of the Rous sarcoma virus (RSV) (Yamamoto et al., *Cell*, 22:787 (1980)); the herpes simplex virus (HSV) thymidine kinase promoter (Wagner et al., *Proc. Nat. Acad. Sci. USA*, 78: 1441 (1981)); the adenovirus promoter (Yamada et al., *Proc. Nat. Acad. Sci. USA*, 82: 3567 (1985)).

The RetL genes may also be introduced by specific viral vectors for use in gene transfer systems which are now well established. See for example: Madzak et al., *J. Gen. Virol.*, 73: 1533-36, 1992 (papovavirus SV40); Berkner et al., *Curr. Top. Microbiol. Immunol.*, 158: 39-61, 1992 (adenovirus); Hofmann et al., *Proc. Natl. Acad. Sci.* 92: 10099-10103, 1995 (baculovirus); Moss et al., *Curr. Top. Microbiol. Immunol.*, 158: 25-38, 1992 (vaccinia virus); Muzyczka, *Curr. Top. Microbiol. Immunol.*, 158: 97-123, 1992 ( adeno-associated virus); Margulskes, *Curr. Top. Microbiol. Immunol.*, 158: 67-93, 1992 (herpes simplex virus (HSV) and Epstein-Barr virus (HBV)); Miller, *Curr. Top. Microbiol. Immunol.*, 158: 1-24, 1992 (retrovirus); Brandyopadhyay et al., *Mol. Cell. Biol.*, 4: 749-754, 1984 (retrovirus); Miller et al., *Nature*, 357: 455-450, 1992 (retrovirus); Anderson, *Science*, 256: 808-813, 1992 (retrovirus), *Current Protocols in Molecular Biology*: Sections 9.10-9.14 (Ausubel et al., Eds.), Greene Publishing Associates, 1989, all of which are incorporated herein by reference.

Preferred vectors are DNA viruses that include adenoviruses (preferably Ad-2 or Ad-5 based vectors), baculovirus, herpes viruses (preferably herpes simplex virus based vectors), and parvoviruses (preferably "defective" or non-autonomous parvovirus based vectors, more preferably adeno-associated virus based vectors, most preferably AAV-2 based vectors). See,

e.g., Ali et al., Gene Therapy 1: 367-384, 1994; U.S. Patent 4,797,368 and 5,399,346 and discussion below.

The choice of a particular vector system for transferring, for instance, a RetL sequence will depend on a variety of factors. One important factor is the nature of the target cell population. Although retroviral vectors have been extensively studied and used in a number of gene therapy applications, they are generally unsuited for infecting cells that are not dividing but may be useful in cancer therapy since they only integrate and express their genes in replicating cells. They are useful for ex vivo approaches and are attractive in this regard due to their stable integration into the target cell genome.

Adenoviruses are eukaryotic DNA viruses that can be modified to efficiently deliver a therapeutic or reporter transgene to a variety of cell types. The general adenoviruses types 2 and 5 (Ad2 and Ad5, respectively), which cause respiratory disease in humans, are currently being developed for gene therapy of Duchenne Muscular Dystrophy (DMD) and Cystic Fibrosis (CF). Both Ad2 and Ad5 belong to a subclass of adenovirus that are not associated with human malignancies. Adenovirus vectors are capable of providing extremely high levels of transgene delivery to virtually all cell types, regardless of the mitotic state. High titers ( $10^{13}$  plaque forming units/ml) of recombinant virus can be easily generated in 293 cells (an adenovirus-transformed, complementation human embryonic kidney cell line: ATCC CRL1573) and cryo-stored for extended periods without appreciable losses. The efficacy of this system in delivering a therapeutic transgene *in vivo* that complements a genetic imbalance has been demonstrated in animal models of various disorders. See Y. Watanabe, Atherosclerosis, 36: 261-268 (1986); K. Tanzawa et al, FEBS Letters, 118(1):81-84 (1980); J.L. Golastan et al, New Engl. J. Med., 309 (11983): 288-296 (1983); S. Ishibashi et al, J. Clin. Invest., 92: 883-893 (1993); and S. Ishibashi et al, J. Clin. Invest., 93: 1889-1893 (1994), all of which are incorporated herein by reference. Indeed, recombinant replication defective adenovirus encoding a cDNA for the cystic fibrosis transmembrane regulator (CFTR) has been approved for use in at least two human CF clinical trials. See, e.g., J. Wilson, Nature, 365: 691-692 (Oct., 21, 1993). Further support of the safety of recombinant adenoviruses for gene therapy is the extensive experience of live adenovirus vaccines in human populations.

Human adenoviruses are comprised of a linear, approximately 36 kb double-stranded DNA genome, which is divided into 100 map units (m.u.), each of which is 360 bp in length. The

DNA contains short inverted terminal repeats (ITR) at each end of the genome that are required for viral DNA replication. The gene products are organized into early (E1 through E4) and late (L1 through L5) regions, based on expression before or after the initiation of viral DNA synthesis. See, e.g., Horwitz, *Virology*, 2d edit., ed. B.N. Fields, Raven Press Ltd., New York (1990).

The first-generation recombinant, replication-deficient adenoviruses which have been developed for gene therapy of DMD and other inherited disorders contain deletions of the entire E1a and part of the E1b regions. This replication-defective virus is grown in 293 cells containing a functional adenovirus E1a gene which provides a transacting E1a protein. E1-deleted viruses are capable of replicating and producing infectious virus in the 293 cells, which provide E1a and E1b region gene products in *trans*. The resulting virus is capable of infecting many cell types and can express the introduced gene (providing it carries its own promoter), but cannot replicate in a cell that does not carry the E1 region DNA unless the cell is infected at a very high multiplicity of infection. Adenoviruses have the advantage that they have a broad host range, can infect quiescent or terminally differentiated cells such as neurons, and appear essentially non-oncogenic. Adenoviruses do not appear to integrate in to the host genome. Because they exist extrachromasomally, the risk of insertional mutagenesis is greatly reduced. Ali et al., *supra*, at 373. Recombinant adenoviruses (rAdV) produce very high titers, the viral particles are moderately stable, expression levels are high, and a wide range of cells can be infected. Their natural host cells are airway epithelium, so they are useful for therapy of lung cancers.

Baculovirus-mediated transfer has several advantages. Baculoviral gene transfer can occur in replicating and nonreplicating cells, and can occur in renal cells, as well as in hepatocytes, neural cells, spleen, skin, and muscle. Baculovirus is non-replicating and nonpathogenic in mammalian cells. Humans lack pre-existing antibodies to recombinant baculovirus which could block infection. In addition, baculovirus is capable of incorporating and transducing very large DNA inserts.

Adeno-associated viruses (AAV) have also been employed as vectors for somatic gene therapy. AAV is a small, single-stranded (ss) DNA virus with a simple genomic organization (4.7 kb) that makes it an ideal substrate for genetic engineering. Two open reading frames encode a series of *rep* and *cap* polypeptides. *Rep* polypeptides (*rep78*, *rep68*, *rep 62* and *rep 40*)

are involved in replication, rescue and integration of the AAV genome. The cap proteins (VP1, VP2 and VP3) form the virion capsid. Flanking the *rep* and *cap* open reading frames at the 5' and 3' ends are 145 bp inverted terminal repeats (ITRs), the first 125 bp of which are capable of forming Y- or T-shaped duplex structures. Of importance for the development of AAV vectors, the entire *rep* and *cap* domains can be excised and replaced with a therapeutic or reporter transgene. See B.J. Carter, in Handbook of Parvoviruses, ed., P. Tijsser, CRC Press, pp. 155-168 (1990). It has been shown that the ITRs represent the minimal sequence required for replication, rescue, packaging, and integration of the AAV genome.

The AAV life cycle is biphasic, composed of both latent and lytic episodes. During a latent infection, AAV virions enter a cell as an encapsulated ssDNA, and shortly thereafter are delivered to the nucleus where the AAV DNA stably integrates in to a host chromosome without the apparent need for host cell division. In the absence of a helper virus, the integrated AAV genome remains latent but capable of being activated and rescued. The lytic phase of the life cycle begins when a cell harboring an AAV provirus is challenged with a secondary infection by a herpesvirus or adenovirus which encodes helper functions that are recruited by AAV to aid in its excision from host chromatin (B.J. Carter, supra). The infecting parental ssDNA is expanded to duplex replicating form (RF) DNAs in a *rep* dependent manner. The rescued AAV genomes are packaged into preformed protein capsids (icosahedral symmetry approximately 20 nm in diameter) and released as infectious virions that have packaged either + or - ssDNA genomes following cell lysis.

Adeno-associated viruses (AAV) have significant potential in gene therapy. The viral particles are very stable and recombinant AAVs (rAAV) have "drug-like" characteristics in that rAAV can be purified by pelleting or by CsCl gradient banding. They are heat stable and can be lyophilized to a powder and rehydrated to full activity. Their DNA stably integrates into host chromosomes so expression is long-term. Their host range is broad and AAV causes no known disease so that the recombinant vectors are non-toxic.

Once introduced into a target cell, sequences of interest can be identified by conventional methods such as nucleic acid hybridization using probes comprising sequences that are homologous/complementary to the inserted gene sequences of the vector. In another approach, the sequence(s) may be identified by the presence or absence of a "marker" gene function (e.g,



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thymidine kinase activity, antibiotic resistance, and the like) caused by introduction of the expression vector into the target cell.

### **Formulations and Administration**

The compounds of the invention may be administered in any manner which is medically acceptable. This may include injections, by parenteral routes such as intravenous, intravascular, intraarterial, subcutaneous, intramuscular, intratumor, intraperitoneal, intraventricular, intraepidural, or others as well as oral, nasal, ophthalmic, rectal, or topical. Sustained release administration is also specifically included in the invention, by such means as depot injections or erodible implants. Localized delivery is particularly contemplated, by such means as delivery via a catheter to one or more arteries, such as the renal artery or a vessel supplying a localized tumor.

The term "pharmaceutically acceptable carrier" means one or more organic or inorganic ingredients, natural or synthetic, with which the mutant proto-oncogene or mutant oncoprotein is combined to facilitate its application. A suitable carrier includes sterile saline although other aqueous and non-aqueous isotonic sterile solutions and sterile suspensions known to be pharmaceutically acceptable are known to those of ordinary skill in the art. In this regard, the term "carrier" encompasses liposomes and the HIV-1 tat protein (See Chen et al., Anal. Biochem. 227: 168-175, 1995) as well as any plasmid and viral expression vectors. An "effective amount" refers to that amount which is capable of ameliorating or delaying progression of the diseased, degenerative or damaged condition. An effective amount can be determined on an individual basis and will be based, in part, on consideration of the symptoms to be treated and results sought. An effective amount can be determined by one of ordinary skill in the art employing such factors and using no more than routine experimentation.

The liposome system may be any variety of unilamellar vesicles, multilamellar vesicles, or stable plurilamellar vesicles, and may be prepared and administered according to methods well known to those of skill in the art, for example in accordance with the teachings of United States Patents 5,169,637, 4,762,915, 5,000,958 or 5,185,154. In addition, it may be desirable to express the novel polypeptides of this invention, as well as other selected polypeptides, as lipoproteins, in order to enhance their binding to liposomes. As an example, treatment of human acute renal failure with liposome-encapsulated RetL may be performed in vivo by introducing a

RetL into cells in need of such treatment using liposomes . The liposomes can be delivered via catheter to the renal artery. The recombinant RetL protein is purified, for example, from CHO cells by immunoaffinity chromatography or any other convenient method, then mixed with liposomes and incorporated into them at high efficiency. The encapsulated protein may be tested  
5 in vitro for any effect on stimulating cell growth.

This invention also contemplates that the novel polypeptide of this invention may be administered to an animal via liposome delivery system in order to enhance their stability and/or immunogenicity. Delivery of the novel polypeptides via liposomes may be particularly advantageous because the liposome may be internalized by phagocytic cells in the treated  
10 animal. Such cells, upon ingesting the liposomal membrane and subsequently present the polypeptides to the immune system in conjunction with other molecules required to elicit a strong immune response.

Any of the novel RetL polypeptides of this invention may be used in the form of a pharmaceutically acceptable salt. Suitable acids and bases which are capable of forming salts  
15 with the polypeptides of the present invention are well known to those of skill in the art, and include inorganic and organic acids and bases.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious to one skilled in the art that certain changes and modifications may be practiced within the scope of the invention, as  
20 limited only by the scope of the appended claims.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: doherthy, jodi
- (ii) TITLE OF INVENTION: biogen patent application title
- ~~(iii) NUMBER OF SEQUENCES: 21~~
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Biogen, Inc.
  - (B) STREET: 14 Cambridge Center
  - (C) CITY: Cambridge
  - (D) STATE: MA
  - (E) COUNTRY: USA
  - (F) ZIP: 02142
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE: 07-MAY-1997
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: US 08/999,999
  - (B) FILING DATE: 01-JAN-1996
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Levine, Leslie M.
  - (B) REGISTRATION NUMBER: 35,245
  - (C) REFERENCE/DOCKET NUMBER: A008 PCT CIP
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 617-679-2400
  - (B) TELEFAX: 617-679-2838

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 3616 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 257..1660

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCGGCCGCAG GTTGGGTCGG AACTGAACCC CTGAAAGCGG GTCCGCCTCC CGCCCTCGCG	60
CCCCCCCCGA TCTGAGTCGC TGGCGGCGGT GGGCGGCAGA GCGACGGGGA GTCTGCTCTC	120
ACCCCTGGATG GAGCTGAACT TTGAGTGGCC AGAGGAGCGC AGTCGCCCCG GGATCGCTGC	180
ACGCTGAGCT CTCTCCCCGA GACCGGGCGG CGGCTTTGGA TTTTGGGGGG GCGGGGACCA	240
GCTGCGCGGC GGCACC ATG TTC CTA GCC ACT CTG TAC TTC GCG CTG CCA	289
Met Phe Leu Ala Thr Leu Tyr Phe Ala Leu Pro	
1 5 10	
CTC CTG GAT TTG CTG ATG TCC GCC GAG GTG AGT GGT GGA GAC CGT CTG	337
Leu Leu Asp Leu Leu Met Ser Ala Glu Val Ser Gly Gly Asp Arg Leu	
15 20 25	
GAC TGT GTG AAA GCC AGC GAT CAG TGC CTG AAG GAA CAG AGC TGC AGC	385
Asp Cys Val Lys Ala Ser Asp Gln Cys Leu Lys Glu Gln Ser Cys Ser	
30 35 40	
ACC AAG TAC CGC ACA CTA AGG CAG TGC GTG GCG GGC AAG GAA ACC AAC	433
Thr Lys Tyr Arg Thr Leu Arg Gln Cys Val Ala Gly Lys Glu Thr Asn	
45 50 55	
TTC AGC CTG ACA TCC GGC CTT GAG GCC AAG GAT GAG TGC CGT AGC GCC	481
Phe Ser Leu Thr Ser Gly Leu Glu Ala Lys Asp Glu Cys Arg Ser Ala	
60 65 70 75	
ATG GAG GCC TTG AAG CAG AAG TCT CTG TAC AAC TGC CGC TGC AAG CGG	529
Met Glu Ala Leu Lys Gln Lys Ser Leu Tyr Asn Cys Arg Cys Lys Arg	
80 85 90	
GGC ATG AAG AAA GAG AAG AAT TGT CTG CGT ATC TAC TGG AGC ATG TAC	577
Gly Met Lys Lys Glu Lys Asn Cys Leu Arg Ile Tyr Trp Ser Met Tyr	
95 100 105	
CAG AGC CTG CAG GGA AAT GAC CTC CTG GAA GAT TCC CCG TAT GAG CCG	625
Gln Ser Leu Gln Gly Asn Asp Leu Leu Glu Asp Ser Pro Tyr Glu Pro	
110 115 120	
GTT AAC AGC AGG TTG TCA GAT ATA TTC CGG GCA GTC CCG TTC ATA TCA	673
Val Asn Ser Arg Leu Ser Asp Ile Phe Arg Ala Val Pro Phe Ile Ser	
125 130 135	

47

GAT GTT TTC CAG CAA GTG GAA CAC ATT TCC AAA GGG AAC AAC TGC CTG Asp Val Phe Gln Gln Val Glu His Ile Ser Lys Gly Asn Asn Cys Leu 140 145 150 155	721
GAC GCA GCC AAG GCC TGC AAC CTG GAC GAC ACC TGT AAG AAG TAC AGG Asp Ala Ala Lys Ala Cys Asn Leu Asp Asp Thr Cys Lys Lys Tyr Arg 160 165 170	769
TCG GCC TAC ATC ACC CCC TGC ACC ACC AGC ATG TCC AAC GAG GTC TGC <del>Ser Ala Tyr Ile Thr Pro Cys Thr Thr Ser Met Ser Asn Glu Val Cys</del> 175 180 185	817
AAC CGC CGT AAG TGC CAC AAG GCC CTC AGG CAG TTC TTC GAC AAG GTT Asn Arg Arg Lys Cys His Lys Ala Leu Arg Gln Phe Phe Asp Lys Val 190 195 200	865
CCG GCC AAG CAC AGC TAC GGG ATG CTC TTC TGC TCC TGC CGG GAC ATC Pro Ala Lys His Ser Tyr Gly Met Leu Phe Cys Ser Cys Arg Asp Ile 205 210 215	913
GCC TGC ACC GAG CGG CGG CGA CAG ACT ATC GTC CCC GTG TGC TCC TAT Ala Cys Thr Glu Arg Arg Arg Gln Thr Ile Val Pro Val Cys Ser Tyr 220 225 230 235	961
GAA GAA CGA GAG AGG CCC AAC TGC CTG AGT CTG CAA GAC TCC TGC AAG Glu Glu Arg Glu Arg Pro Asn Cys Leu Ser Leu Gln Asp Ser Cys Lys 240 245 250	1009
ACC AAT TAC ATC TGC AGA TCT CGC CTT GCA GAT TTT TTT ACC AAC TGC Thr Asn Tyr Ile Cys Arg Ser Arg Leu Ala Asp Phe Phe Thr Asn Cys 255 260 265	1057
CAG CCA GAG TCA AGG TCT GTC AGC AAC TGT CTT AAG GAG AAC TAC GCA Gln Pro Glu Ser Arg Ser Val Ser Asn Cys Leu Lys Glu Asn Tyr Ala 270 275 280	1105
GAC TGC CTC CTG GCC TAC TCG GGA CTG ATT GGC ACA GTC ATG ACT CCC Asp Cys Leu Leu Ala Tyr Ser Gly Leu Ile Gly Thr Val Met Thr Pro 285 290 295	1153
AAC TAC GTA GAC TCC AGC AGC CTC AGC GTG GCA CCA TGG TGT GAC TGC Asn Tyr Val Asp Ser Ser Ser Leu Ser Val Ala Pro Trp Cys Asp Cys 300 305 310 315	1201
AGC AAC AGC GGC AAT GAC CTG GAA GAC TGC TTG AAA TTT CTG AAT TTT Ser Asn Ser Gly Asn Asp Leu Glu Asp Cys Leu Lys Phe Leu Asn Phe 320 325 330	1249
TTT AAG GAC AAT ACT TGT CTC AAA AAT GCA ATT CAA GCC TTT GGC AAT Phe Lys Asp Asn Thr Cys Leu Lys Asn Ala Ile Gln Ala Phe Gly Asn 335 340 345	1297
GGC TCA GAT GTG ACC ATG TGG CAG CCA GCC CCT CCA GTC CAG ACC ACC Gly Ser Asp Val Thr Met Trp Gln Pro Ala Pro Pro Val Gln Thr Thr 350 355 360	1345

48

ACT GCC ACC ACT ACC ACT GCC TTC CGG GTC AAG AAC AAG CCT CTG GGG Thr Ala Thr Thr Thr Thr Thr Ala Phe Arg Val Lys Asn Lys Pro Leu Gly 365 370 375	1393
CCA GCA GGG TCT GAG AAT GAG ATC CCC ACA CAC GTT TTA CCA CCC TGT Pro Ala Gly Ser Glu Asn Glu Ile Pro Thr His Val Leu Pro Pro Cys 380 385 390 395	1441
GCG AAT TTG CAG GCT CAG AAG CTG AAA TCC AAT GTG TCG GGT AGC ACA Ala Asn Leu Gln Ala Gln Lys Leu Lys Ser Asn Val Ser Gly Ser Thr 400 405 410	1489
CAC CTC TGT CTT TCT GAT AGT GAT TTC GGA AAG GAT GGT CTC GCT GGT His Leu Cys Leu Ser Asp Ser Asp Phe Gly Lys Asp Gly Leu Ala Gly 415 420 425	1537
GCC TCC AGC CAC ATA ACC ACA AAA TCA ATG GCT GCT CCT CCC AGC TGC Ala Ser Ser His Ile Thr Thr Lys Ser Met Ala Ala Pro Pro Ser Cys 430 435 440	1585
AGT CTG AGC TCA CTG CCG GTG CTG ATG CTC ACC GCC CTT GCT GCC CTG Ser Leu Ser Ser Leu Pro Val Leu Met Leu Thr Ala Leu Ala Ala Leu 445 450 455	1633
TTA TCT GTA TCG TTG GCA GAA ACG TCG TAGCTGCATC CGGGAAAACA Leu Ser Val Ser Leu Ala Glu Thr Ser 460 465	1680
GTATGAAAAG ACAAAGAGA ACCAAGTATT CTGTCCCTGT CCTCTTGAT ATCTGAAAAT	1740
CCAGTTTTAA AAGCTCCGTT GAGAAGCAGT TTCACCCAAC TGGAACCTTT TCCTTGTTTT	1800
TAAGAAAGCT TGTGGCCCTC AGGGGCTTCT GTTGAAGAAC TGCTACAGGG CTAATTCCAA	1860
ACCCATAAGG CTCTGGGGCG TGGTGC GGCT TAAGGGGACC ATTTGCACCA TGTAAGCAA	1920
GCTGGGCTTA TCATGTGTTT GATGGTGAGG ATGGTAGTGG TGATGATGAT GGTAATTTTA	1980
ACAGCTTGAA CCCTGTTCTC TCTACTGGTT AGGAACAGGA GATACTATTG ATAAAGATTC	2040
TTCCATGTCT TACTCAGCAG CATTGCCTTC TGAAGACAGG CCCGCAGCCT AGTGTGAATG	2100
ACAAGTGGAG GTTGGCCTCA AGAGTGGACT TGGCAGACTC TACCTTG TAG TAATGTTTAC	2160
CTTTCCGTGT ATGGTCTCCA CAGAGTGTTT ATGTATTTAC AGACTGTTCT GTGATCCCCC	2220
AACAACAACA ACCACAAATT CCTTGGTCAC CTCCAAATGT AACCGGTCCT TTAGCCCAGT	2280
AGAGGAGGGT GGGTGTGGCC CTGGCACAGC TCCCGGATTG TTGATGGGCA CTCTCCTGAG	2340
CTTTGCTTGA GTGAGAAGCT GAATGTAGCT GAAAATCAAC TCTTCTTACA CTTCTTACTG	2400
CTTCGTTTAC TTACGAGGTC ACATATAGAA CAAACATCAC CAACTATTAG CTTACCGTTA	2460

49

GCTTCCCAAC TATTAGCTTT CTATGTTTTG AAAGCAGTGT TGCTGACCCC ATGTTTTTAAT	2520
GATGGTTTAA TACATGCAGC CCTTTCCTCT CATCGGTAAC ACTAGCTCCA ACATCAACTT	2580
CATGCATGTG GCTCTCAAAA GCAGGCCCCA AGAAGCCCAG TTCTTTAGGA GAAAGCTGCG	2640
TCCTGTTTCT GTGGACAGGC AGGAGGAAAC AGAGCAGCCT GCCCGTGGTG TCTTTATCTG	2700
TTTTGAAATC AAGGCTGCCT GTGTGTAAGG AATGGTTCAA TTCTTATAAA GGGTGCCACT	2760
GTTGATGCCA CAACTGGCAG TTGGTCTAGC TCCAGGACAC CGGTTTCCAT GTTGCCCTGGC	2820
AGAGACAGCT TTGATTGGGA CTGGCTGGCC ACAAGGGATG GGATGAAGAT GTGCTGCCCT	2880
CTCTTTCAAA GTTGAGCCCT GCCAGGGCAC ATAGAAGCAT CTTTGCTCCT GACCACAACG	2940
TAGAACAGCT TGGATTCAAG GTCATCAAGC GTCTCCTGTA CATTGCTCTG TGACCTTCAT	3000
AACAGACTGT CCCGCACAAA AGGAACGGCA GTTTATGGAT CTAGAGTGGG AGCACAGGGT	3060
CTGGAAAGGT GAACCGATTG GCAAAATACA CAGAACAGGA GGGAGAGTCT CAAGCCGAGA	3120
CATCTTGCTT ACTAGCCACA CACCATCTCC TGGAGCCCTC CTCCTGACCT GGGCAGACCC	3180
TTAGGTGTAT ATCTAAAGAC CTCTTCAATG TTCAGGTTCA GAATCTGTAA ATGGTTGCGT	3240
CCTGGCACCC ATTCCTGAAA ACTGAACAAA GGAGAGGATA TCTTTCCTCC ATTGAGCCCT	3300
GAAAGTATGA CTGGCTTCTC ACCCTCCCAC AGAGCAGGGA GCCCTGGTGC ACACAGTCTC	3360
CTGATATCCT CCCTGCTCTT TGAGGTTTGC CTTGGGAGAA AATGATTCAC CTCGGGAGGG	3420
GACGCTTTGG TGTCTGAAGT ACGTTTATAT CGAAATGTTA ATGAATACCC ATGTAAAATA	3480
CTCAATAGCC ACCTTCTTC CCTTCACAAT GTTTTCGAGG GGAATGCATC CAACATCCAA	3540
GTGTACCTGG TCAGTGGGAA GTTCCATGAA GACTCATACA TTGAATAAAC ATATTCGATG	3600
TGCCGAAAGC GGCCGC	3616

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 468 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Phe	Leu	Ala	Thr	Leu	Tyr	Phe	Ala	Leu	Pro	Leu	Leu	Asp	Leu	Leu
1				5					10					15	

Met Ser Ala Glu Val Ser Gly Gly Asp Arg Leu Asp Cys Val Lys Ala  
 20 25 30  
 Ser Asp Gln Cys Leu Lys Glu Gln Ser Cys Ser Thr Lys Tyr Arg Thr  
 35 40 45  
 Leu Arg Gln Cys Val Ala Gly Lys Glu Thr Asn Phe Ser Leu Thr Ser  
 50 55 60  
 Gly Leu Glu Ala Lys Asp Glu Cys Arg Ser Ala Met Glu Ala Leu Lys  
 65 70 75 80  
 Gln Lys Ser Leu Tyr Asn Cys Arg Cys Lys Arg Gly Met Lys Lys Glu  
 85 90 95  
 Lys Asn Cys Leu Arg Ile Tyr Trp Ser Met Tyr Gln Ser Leu Gln Gly  
 100 105 110  
 Asn Asp Leu Leu Glu Asp Ser Pro Tyr Glu Pro Val Asn Ser Arg Leu  
 115 120 125  
 Ser Asp Ile Phe Arg Ala Val Pro Phe Ile Ser Asp Val Phe Gln Gln  
 130 135 140  
 Val Glu His Ile Ser Lys Gly Asn Asn Cys Leu Asp Ala Ala Lys Ala  
 145 150 155 160  
 Cys Asn Leu Asp Asp Thr Cys Lys Lys Tyr Arg Ser Ala Tyr Ile Thr  
 165 170 175  
 Pro Cys Thr Thr Ser Met Ser Asn Glu Val Cys Asn Arg Arg Lys Cys  
 180 185 190  
 His Lys Ala Leu Arg Gln Phe Phe Asp Lys Val Pro Ala Lys His Ser  
 195 200 205  
 Tyr Gly Met Leu Phe Cys Ser Cys Arg Asp Ile Ala Cys Thr Glu Arg  
 210 215 220  
 Arg Arg Gln Thr Ile Val Pro Val Cys Ser Tyr Glu Glu Arg Glu Arg  
 225 230 235 240  
 Pro Asn Cys Leu Ser Leu Gln Asp Ser Cys Lys Thr Asn Tyr Ile Cys  
 245 250 255  
 Arg Ser Arg Leu Ala Asp Phe Phe Thr Asn Cys Gln Pro Glu Ser Arg  
 260 265 270  
 Ser Val Ser Asn Cys Leu Lys Glu Asn Tyr Ala Asp Cys Leu Leu Ala  
 275 280 285  
 Tyr Ser Gly Leu Ile Gly Thr Val Met Thr Pro Asn Tyr Val Asp Ser  
 290 295 300



Ser Ser Leu Ser Val Ala Pro Trp Cys Asp Cys Ser Asn Ser Gly Asn  
305 310 315 320

Asp Leu Glu Asp Cys Leu Lys Phe Leu Asn Phe Phe Lys Asp Asn Thr  
325 330 335

Cys Leu Lys Asn Ala Ile Gln Ala Phe Gly Asn Gly Ser Asp Val Thr  
340 345 350

Met Trp Gln Pro Ala Pro Pro Val Gln Thr Thr Thr Ala Thr Thr Thr  
355 360 365

Thr Ala Phe Arg Val Lys Asn Lys Pro Leu Gly Pro Ala Gly Ser Glu  
370 375 380

Asn Glu Ile Pro Thr His Val Leu Pro Pro Cys Ala Asn Leu Gln Ala  
385 390 395 400

Gln Lys Leu Lys Ser Asn Val Ser Gly Ser Thr His Leu Cys Leu Ser  
405 410 415

Asp Ser Asp Phe Gly Lys Asp Gly Leu Ala Gly Ala Ser Ser His Ile  
420 425 430

Thr Thr Lys Ser Met Ala Ala Pro Pro Ser Cys Ser Leu Ser Ser Leu  
435 440 445

Pro Val Leu Met Leu Thr Ala Leu Ala Ala Leu Leu Ser Val Ser Leu  
450 455 460

Ala Glu Thr Ser  
465

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 39 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AAGGAAAAAA GCGGCCGCCA TGGCGAAGGC GACGTCCGG

39

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 33 base pairs
  - (B) TYPE: nucleic acid

52

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AGTTTTGTCTG ACCGTGCGGC ACAGCTCGTC GCA

33

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AGTTTTGTCTG ACCGTGCGGC ACAGCGCATC ACA

33

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1926 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 10..1920

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GCGGCCGCC ATG GCG AAG GCG ACG TCC GGC GCC GCA GGG CTG GGG CTG  
Met Ala Lys Ala Thr Ser Gly Ala Ala Gly Leu Gly Leu  
470 475 480

48

AAG CTG TTT TTG CTG CTG CCG CTA CTG GGA GAA GCC CCG CTG GGT CTC  
Lys Leu Phe Leu Leu Leu Pro Leu Leu Gly Glu Ala Pro Leu Gly Leu  
485 490 495

96

53

TAC	TTC	TCA	AGG	GAT	GCT	TAC	TGG	GAG	AGG	CTG	TAT	GTG	GAC	CAG	CCA	144
Tyr	Phe	Ser	Arg	Asp	Ala	Tyr	Trp	Glu	Arg	Leu	Tyr	Val	Asp	Gln	Pro	
		500					505					510				
GCT	GGC	ACA	CCT	CTG	CTC	TAT	GTC	CAT	GCC	CTA	CGG	GAT	GCC	CCT	GGA	192
Ala	Gly	Thr	Pro	Leu	Leu	Tyr	Val	His	Ala	Leu	Arg	Asp	Ala	Pro	Gly	
	515					520				525						
GAA	GTG	CCC	AGC	TTC	CGC	CTG	GGC	CAG	TAT	CTC	TAT	GGC	GTC	TAC	CGC	240
<del>Glu</del>	<del>Val</del>	<del>Pro</del>	<del>Ser</del>	<del>Phe</del>	<del>Arg</del>	<del>Leu</del>	<del>Gly</del>	<del>Gln</del>	<del>Tyr</del>	<del>Leu</del>	<del>Tyr</del>	<del>Gly</del>	<del>Val</del>	<del>Tyr</del>	<del>Arg</del>	
530					535				540						545	
ACG	CGT	CTG	CAT	GAG	AAT	GAC	TGG	ATC	CAC	ATC	GAT	GCG	GGC	ACT	GGC	288
Thr	Arg	Leu	His	Glu	Asn	Asp	Trp	Ile	His	Ile	Asp	Ala	Gly	Thr	Gly	
				550					555					560		
CTC	CTC	TAC	CTC	AAT	CAG	AGC	CTG	GAC	CAT	AGT	TCC	TGG	GAG	CAG	CTC	336
Leu	Leu	Tyr	Leu	Asn	Gln	Ser	Leu	Asp	His	Ser	Ser	Trp	Glu	Gln	Leu	
			565					570					575			
AGC	ATC	CGA	AAT	GGC	GGC	TTC	CCC	TTG	CTC	ACC	GTC	TTC	CTC	CAG	GTC	384
Ser	Ile	Arg	Asn	Gly	Gly	Phe	Pro	Leu	Leu	Thr	Val	Phe	Leu	Gln	Val	
		580					585				590					
TTC	CTG	GGG	TCC	ACA	GCC	CAG	AGA	GAG	GGA	GAG	TGT	CAT	TGG	CCA	GGC	432
Phe	Leu	Gly	Ser	Thr	Ala	Gln	Arg	Glu	Gly	Glu	Cys	His	Trp	Pro	Gly	
	595					600					605					
TGT	GCC	CGT	GTG	TAC	TTC	TCC	TTC	ATC	AAC	GAC	ACC	TTC	CCA	AAT	TGT	480
Cys	Ala	Arg	Val	Tyr	Phe	Ser	Phe	Ile	Asn	Asp	Thr	Phe	Pro	Asn	Cys	
610					615					620					625	
AGC	TCC	TTC	AAA	GCC	CGG	GAT	CTC	TGC	ACC	CCA	GAG	ACG	GGT	GTG	TCC	528
Ser	Ser	Phe	Lys	Ala	Arg	Asp	Leu	Cys	Thr	Pro	Glu	Thr	Gly	Val	Ser	
				630					635					640		
TTC	CGC	ATC	AGG	GAG	AAC	AGG	CCC	CCT	GGC	ACC	TTC	TAC	CAG	TTC	CGC	576
Phe	Arg	Ile	Arg	Glu	Asn	Arg	Pro	Pro	Gly	Thr	Phe	Tyr	Gln	Phe	Arg	
			645					650					655			
ATG	CTA	CCT	GTG	CAG	TTC	CTT	TGT	CCT	AAC	ATC	AGT	GTG	AAG	TAC	AAA	624
Met	Leu	Pro	Val	Gln	Phe	Leu	Cys	Pro	Asn	Ile	Ser	Val	Lys	Tyr	Lys	
		660					665					670				
CTC	TTA	GAA	GGG	GAC	GGT	CTG	CCC	TTC	CGT	TGT	GAC	CCC	GAC	TGT	CTG	672
Leu	Leu	Glu	Gly	Asp	Gly	Leu	Pro	Phe	Arg	Cys	Asp	Pro	Asp	Cys	Leu	
	675					680					685					
GAG	GTG	AGC	ACG	CGG	TGG	GCA	CTG	GAT	CGG	GAG	CTT	CAG	GAG	AAG	TAT	720
Glu	Val	Ser	Thr	Arg	Trp	Ala	Leu	Asp	Arg	Glu	Leu	Gln	Glu	Lys	Tyr	
690					695				700					705		
GTG	CTG	GAG	GCT	GAG	TGC	GCA	GTG	GCA	GGC	CCT	GGA	GCC	AAC	AAG	GAG	768
Val	Leu	Glu	Ala	Glu	Cys	Ala	Val	Ala	Gly	Pro	Gly	Ala	Asn	Lys	Glu	
				710					715					720		

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AAG GTG GCC GTG TCC TTC CCG GTG ACG GTG TAT GAT GAA GAC GAC TCC Lys Val Ala Val Ser Phe Pro Val Thr Val Tyr Asp Glu Asp Asp Ser	816
725 730 735	
CCG CCC ACC TTC TCC GGA GGT GTG GGC ACC GCC AGT GCT GTG GTG GAG Pro Pro Thr Phe Ser Gly Gly Val Gly Thr Ala Ser Ala Val Val Glu	864
740 745 750	
TTT AAG CGG AAG GAG GGC ACT GTG GTA GCC ACT CTG CAG GTG TTT GAT Phe Lys Arg Lys Glu Gly Thr Val Val Ala Thr Leu Gln Val Phe Asp	912
755 760 765	
GCA GAT GTG GTG CCA GCA TCT GGG GAG CTG GTG AGG CGG TAC ACA AGC Ala Asp Val Val Pro Ala Ser Gly Glu Leu Val Arg Arg Tyr Thr Ser	960
770 775 780 785	
ACA CTA CTC TCA GGG GAT TCC TGG GCC CAG CAG ACC TTC CGG GTG GAG Thr Leu Leu Ser Gly Asp Ser Trp Ala Gln Gln Thr Phe Arg Val Glu	1008
790 795 800	
CAC ACA CCC AAC GAG ACC TTG GTC CAG TCC AAC AAC AAC TCC GTG CGG His Thr Pro Asn Glu Thr Leu Val Gln Ser Asn Asn Asn Ser Val Arg	1056
805 810 815	
GCA ACC ATG CAC AAT TAC AAG CTG GTT CTC AAC AGG AGC CTG TCC ATC Ala Thr Met His Asn Tyr Lys Leu Val Leu Asn Arg Ser Leu Ser Ile	1104
820 825 830	
TCA GAG AGC CGA GTC CTG CAG CTA GTA GTC CTG GTC AAT GAC TCA GAC Ser Glu Ser Arg Val Leu Gln Leu Val Val Leu Val Asn Asp Ser Asp	1152
835 840 845	
TTC CAG GGG CCT GGG TCA GGT GTT CTC TTC CTC CAT TTC AAC GTG TCT Phe Gln Gly Pro Gly Ser Gly Val Leu Phe Leu His Phe Asn Val Ser	1200
850 855 860 865	
GTG CTG CCT GTC ACC CTG AAC CTA CCC ATG GCC TAC TCC TTC CCA GTG Val Leu Pro Val Thr Leu Asn Leu Pro Met Ala Tyr Ser Phe Pro Val	1248
870 875 880	
AAT AGG AGA GCC CGC CGT TAT GCC CAG ATT GGG AAA GTT TGC GTG GAG Asn Arg Arg Ala Arg Arg Tyr Ala Gln Ile Gly Lys Val Cys Val Glu	1296
885 890 895	
AAC TGC CAG GAG TTC AGC GGT GTC TCC ATC CAG TAC AAG CTG CAG CCC Asn Cys Gln Glu Phe Ser Gly Val Ser Ile Gln Tyr Lys Leu Gln Pro	1344
900 905 910	
TCC AGC ACC AAC TGC AGT GCC CTA GGT GTG GTC ACC TCA ACA GAA GAC Ser Ser Thr Asn Cys Ser Ala Leu Gly Val Val Thr Ser Thr Glu Asp	1392
915 920 925	

ACC TCA GGG ACC CTA TAT GTA AAT GAC ACG GAG GCC CTG CGG CGA CCT	1440
Thr Ser Gly Thr Leu Tyr Val Asn Asp Thr Glu Ala Leu Arg Arg Pro	
930 935 940 945	
GAG TGT ACC GAG CTT CAG TAC ACA GTG GTA GCC ACT GAC CGG CAG ACC	1488
Glu Cys Thr Glu Leu Gln Tyr Thr Val Val Ala Thr Asp Arg Gln Thr	
950 955 960	
CGC AGG CAG ACC CAA GCT TCG TTA GTC GTC ACA GTG GAG GGG ACA TAC	1536
Arg Arg Gln Thr Gln Ala Ser Leu Val Val Thr Val Glu Gly Thr Tyr	
965 970 975	
ATT GCA GAA GAA GTG GGC TGC CCC AAG TCC TGT GCA GTA AAC AAG AGG	1584
Ile Ala Glu Glu Val Gly Cys Pro Lys Ser Cys Ala Val Asn Lys Arg	
980 985 990	
CGA CCT GAG TGT GAG GAG TGT GGT GGC CTG GGT TCT CCA ACT GGC AGA	1632
Arg Pro Glu Cys Glu Glu Cys Gly Gly Leu Gly Ser Pro Thr Gly Arg	
995 1000 1005	
TGT GAG TGG CGT CAG GGA GAT GGT AAA GGG ATC ACC AGG AAC TTC TCC	1680
Cys Glu Trp Arg Gln Gly Asp Gly Lys Gly Ile Thr Arg Asn Phe Ser	
1010 1015 1020 1025	
ACC TGT TCT CCT AGC ACC AGG ACC TGT CCT GAT GGC CAC TGT GAT GCT	1728
Thr Cys Ser Pro Ser Thr Arg Thr Cys Pro Asp Gly His Cys Asp Ala	
1030 1035 1040	
CTG GAG AGC CGG GAT ATC AAC ATT TGC CCC CAG GAC TGT CTC CGT GGC	1776
Leu Glu Ser Arg Asp Ile Asn Ile Cys Pro Gln Asp Cys Leu Arg Gly	
1045 1050 1055	
CCC ATT GTT GGC GGG CAT GAG CGA GGG GAG CGC CAG GGG ATT AAA GCC	1824
Pro Ile Val Gly Gly His Glu Arg Gly Glu Arg Gln Gly Ile Lys Ala	
1060 1065 1070	
GGC TAT GGC ATC TGC AAC TGT TTC CCT GAT GAG AAG AAG TGC TTC TGC	1872
Gly Tyr Gly Ile Cys Asn Cys Phe Pro Asp Glu Lys Lys Cys Phe Cys	
1075 1080 1085	
GAG CCA GAG GAC AGC CAG GGC CCA TTG TGT GAT GCG CTG TGC CGC ACG	1920
Glu Pro Glu Asp Ser Gln Gly Pro Leu Cys Asp Ala Leu Cys Arg Thr	
1090 1095 1100 1105	
GTCGAC	1926

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 637 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met	Ala	Lys	Ala	Thr	Ser	Gly	Ala	Ala	Gly	Leu	Gly	Leu	Lys	Leu	Phe	1	5	10	15
Leu	Leu	Leu	Pro	Leu	Leu	Gly	Glu	Ala	Pro	Leu	Gly	Leu	Tyr	Phe	Ser	20	25	30	
Arg	Asp	Ala	Tyr	Trp	Glu	Arg	Leu	Tyr	Val	Asp	Gln	Pro	Ala	Gly	Thr	35	40	45	
Pro	Leu	Leu	Tyr	Val	His	Ala	Leu	Arg	Asp	Ala	Pro	Gly	Glu	Val	Pro	50	55	60	
Ser	Phe	Arg	Leu	Gly	Gln	Tyr	Leu	Tyr	Gly	Val	Tyr	Arg	Thr	Arg	Leu	65	70	75	80
His	Glu	Asn	Asp	Trp	Ile	His	Ile	Asp	Ala	Gly	Thr	Gly	Leu	Leu	Tyr	85	90	95	
Leu	Asn	Gln	Ser	Leu	Asp	His	Ser	Ser	Trp	Glu	Gln	Leu	Ser	Ile	Arg	100	105	110	
Asn	Gly	Gly	Phe	Pro	Leu	Leu	Thr	Val	Phe	Leu	Gln	Val	Phe	Leu	Gly	115	120	125	
Ser	Thr	Ala	Gln	Arg	Glu	Gly	Glu	Cys	His	Trp	Pro	Gly	Cys	Ala	Arg	130	135	140	
Val	Tyr	Phe	Ser	Phe	Ile	Asn	Asp	Thr	Phe	Pro	Asn	Cys	Ser	Ser	Phe	145	150	155	160
Lys	Ala	Arg	Asp	Leu	Cys	Thr	Pro	Glu	Thr	Gly	Val	Ser	Phe	Arg	Ile	165	170	175	
Arg	Glu	Asn	Arg	Pro	Pro	Gly	Thr	Phe	Tyr	Gln	Phe	Arg	Met	Leu	Pro	180	185	190	
Val	Gln	Phe	Leu	Cys	Pro	Asn	Ile	Ser	Val	Lys	Tyr	Lys	Leu	Leu	Glu	195	200	205	
Gly	Asp	Gly	Leu	Pro	Phe	Arg	Cys	Asp	Pro	Asp	Cys	Leu	Glu	Val	Ser	210	215	220	
Thr	Arg	Trp	Ala	Leu	Asp	Arg	Glu	Leu	Gln	Glu	Lys	Tyr	Val	Leu	Glu	225	230	235	240
Ala	Glu	Cys	Ala	Val	Ala	Gly	Pro	Gly	Ala	Asn	Lys	Glu	Lys	Val	Ala	245	250	255	
Val	Ser	Phe	Pro	Val	Thr	Val	Tyr	Asp	Glu	Asp	Asp	Ser	Pro	Pro	Thr	260	265	270	

Phe Ser Gly Gly Val Gly Thr Ala Ser Ala Val Val Glu Phe Lys Arg  
275 280 285

Lys Glu Gly Thr Val Val Ala Thr Leu Gln Val Phe Asp Ala Asp Val  
290 295 300

Val Pro Ala Ser Gly Glu Leu Val Arg Arg Tyr Thr Ser Thr Leu Leu  
305 310 315 320

Ser Gly Asp Ser Trp Ala Gln Gln Thr Phe Arg Val Glu His Thr Pro  
325 330 335

Asn Glu Thr Leu Val Gln Ser Asn Asn Asn Ser Val Arg Ala Thr Met  
340 345 350

His Asn Tyr Lys Leu Val Leu Asn Arg Ser Leu Ser Ile Ser Glu Ser  
355 360 365

Arg Val Leu Gln Leu Val Val Leu Val Asn Asp Ser Asp Phe Gln Gly  
370 375 380

Pro Gly Ser Gly Val Leu Phe Leu His Phe Asn Val Ser Val Leu Pro  
385 390 395 400

Val Thr Leu Asn Leu Pro Met Ala Tyr Ser Phe Pro Val Asn Arg Arg  
405 410 415

Ala Arg Arg Tyr Ala Gln Ile Gly Lys Val Cys Val Glu Asn Cys Gln  
420 425 430

Glu Phe Ser Gly Val Ser Ile Gln Tyr Lys Leu Gln Pro Ser Ser Thr  
435 440 445

Asn Cys Ser Ala Leu Gly Val Val Thr Ser Thr Glu Asp Thr Ser Gly  
450 455 460

Thr Leu Tyr Val Asn Asp Thr Glu Ala Leu Arg Arg Pro Glu Cys Thr  
465 470 475 480

Glu Leu Gln Tyr Thr Val Val Ala Thr Asp Arg Gln Thr Arg Arg Gln  
485 490 495

Thr Gln Ala Ser Leu Val Val Thr Val Glu Gly Thr Tyr Ile Ala Glu  
500 505 510

Glu Val Gly Cys Pro Lys Ser Cys Ala Val Asn Lys Arg Arg Pro Glu  
515 520 525

Cys Glu Glu Cys Gly Gly Leu Gly Ser Pro Thr Gly Arg Cys Glu Trp  
530 535 540

Arg Gln Gly Asp Gly Lys Gly Ile Thr Arg Asn Phe Ser Thr Cys Ser  
545 550 555 560

58

Pro	Ser	Thr	Arg	Thr	Cys	Pro	Asp	Gly	His	Cys	Asp	Ala	Leu	Glu	Ser
				565					570					575	
Arg	Asp	Ile	Asn	Ile	Cys	Pro	Gln	Asp	Cys	Leu	Arg	Gly	Pro	Ile	Val
			580					585					590		
Gly	Gly	His	Glu	Arg	Gly	Glu	Arg	Gln	Gly	Ile	Lys	Ala	Gly	Tyr	Gly
		595					600					605			
Ile	Cys	Asn	Cys	Phe	Pro	Asp	Glu	Lys	Lys	Cys	Phe	Cys	Glu	Pro	Glu
	610					615					620				
Asp	Ser	Gln	Gly	Pro	Leu	Cys	Asp	Ala	Leu	Cys	Arg	Thr			
625					630					635					

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1223 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) **FEATURE:**

- (A) NAME/KEY: CDS  
(B) LOCATION: 1..1038

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CTG	CTG	GAG	GAT	TCC	CCA	TAT	GAA	CCA	GTT	AAC	AGC	AGA	TTG	TCA	GAT	48
Leu	Leu	Glu	Asp	Ser	Pro	Tyr	Glu	Pro	Val	Asn	Ser	Arg	Leu	Ser	Asp	
		640					645					650				
ATA	TTC	CGG	GTG	GTC	CCA	TTC	ATA	TCA	GTG	GAG	CAC	ATT	CCC	AAA	GGG	96
Ile	Phe	Arg	Val	Val	Pro	Phe	Ile	Ser	Val	Glu	His	Ile	Pro	Lys	Gly	
		655				660					665					
AAC	AAC	TGC	CTG	GAT	GCA	GCG	AAG	GCC	TGC	AAC	CTC	GAC	GAC	ATT	TGC	144
Asn	Asn	Cys	Leu	Asp	Ala	Ala	Lys	Ala	Cys	Asn	Leu	Asp	Asp	Ile	Cys	
		670			675				680						685	
AAG	AAG	TAC	AGG	TCG	GCG	TAC	ATC	ACC	CCG	TGC	ACC	ACC	AGC	GTG	TCC	192
Lys	Lys	Tyr	Arg	Ser	Ala	Tyr	Ile	Thr	Pro	Cys	Thr	Thr	Ser	Val	Ser	
				690					695					700		
AAC	GAT	GTC	TGC	AAC	CGC	CGC	AAG	TGC	CAC	AAG	GCC	CTC	CGG	CAG	TTC	240
Asn	Asp	Val	Cys	Asn	Arg	Arg	Lys	Cys	His	Lys	Ala	Leu	Arg	Gln	Phe	
			705					710					715			



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TTT	GAC	AAG	GTC	CCG	GCC	AAG	CAC	AGC	TAC	GGA	ATG	CTC	TTC	TGC	TCC	288
Phe	Asp	Lys	Val	Pro	Ala	Lys	His	Ser	Tyr	Gly	Met	Leu	Phe	Cys	Ser	
		720					725					730				
TGC	CGG	GAC	ATC	GCC	TGC	ACA	GAG	CGG	AGG	CGA	CAG	ACC	ATC	GTG	CCT	336
Cys	Arg	Asp	Ile	Ala	Cys	Thr	Glu	Arg	Arg	Arg	Gln	Thr	Ile	Val	Pro	
		735				740					745					
GTG	TGC	TCC	TAT	GAA	GAG	AGG	GAG	AAG	CCC	AAC	TGT	TTG	AAT	TTG	CAG	384
Val	Cys	Ser	Tyr	Glu	Glu	Arg	Glu	Lys	Pro	Asn	Cys	Leu	Asn	Leu	Gln	
		750			755					760					765	
GAC	TCC	TGC	AAG	ACG	AAT	TAC	ATC	TGC	AGA	TCT	CGC	CTT	GCG	GAT	TTT	432
Asp	Ser	Cys	Lys	Thr	Asn	Tyr	Ile	Cys	Arg	Ser	Arg	Leu	Ala	Asp	Phe	
				770					775					780		
TTT	ACC	AAC	TGC	CAG	CCA	GAG	TCA	AGG	TCT	GTC	AGC	AGC	TGT	CTA	AAG	480
Phe	Thr	Asn	Cys	Gln	Pro	Glu	Ser	Arg	Ser	Val	Ser	Ser	Cys	Leu	Lys	
			785					790					795			
GAA	AAC	TAC	GCT	GAC	TGC	CTC	CTC	GCC	TAC	TCG	GGG	CTT	ATT	GGC	ACA	528
Glu	Asn	Tyr	Ala	Asp	Cys	Leu	Leu	Ala	Tyr	Ser	Gly	Leu	Ile	Gly	Thr	
		800						805				810				
GTC	ATG	ACC	CCC	AAC	TAC	ATA	GAC	TCC	AGT	AGC	CTC	AGT	GTG	GCC	CCA	576
Val	Met	Thr	Pro	Asn	Tyr	Ile	Asp	Ser	Ser	Ser	Leu	Ser	Val	Ala	Pro	
		815				820					825					
TGG	TGT	GAC	TGC	AGC	AAC	AGT	GGG	AAC	GAC	CTA	GAA	GAG	TGC	TTG	AAA	624
Trp	Cys	Asp	Cys	Ser	Asn	Ser	Gly	Asn	Asp	Leu	Glu	Glu	Cys	Leu	Lys	
		830			835					840					845	
TTT	TTG	AAT	TTC	TTC	AAG	GAC	AAT	ACA	TGT	CTT	AAA	AAT	GCA	ATT	CAA	672
Phe	Leu	Asn	Phe	Phe	Lys	Asp	Asn	Thr	Cys	Leu	Lys	Asn	Ala	Ile	Gln	
			850						855					860		
GCC	TTT	GGC	AAT	GGC	TCC	GAT	GTG	ACC	GTG	TGG	CAG	CCA	GCC	TTC	CCA	720
Ala	Phe	Gly	Asn	Gly	Ser	Asp	Val	Thr	Val	Trp	Gln	Pro	Ala	Phe	Pro	
			865					870					875			
GTA	CAG	ACC	ACC	ACT	GCC	ACT	ACC	ACC	ACT	GCC	CTC	CGG	GTT	AAG	AAC	768
Val	Gln	Thr	Thr	Thr	Ala	Thr	Thr	Thr	Thr	Ala	Leu	Arg	Val	Lys	Asn	
		880					885					890				
AAG	CCC	CTG	GGG	CCA	GCA	GGG	TCT	GAG	AAT	GAA	ATT	CCC	ACT	CAT	GTT	816
Lys	Pro	Leu	Gly	Pro	Ala	Gly	Ser	Glu	Asn	Glu	Ile	Pro	Thr	His	Val	
		895				900					905					
TTG	CCA	CCG	TGT	GCA	AAT	TTA	CAG	GCA	CAG	AAG	CTG	AAA	TCC	AAT	GTG	864
Leu	Pro	Pro	Cys	Ala	Asn	Leu	Gln	Ala	Gln	Lys	Leu	Lys	Ser	Asn	Val	
		910			915					920					925	
TCG	GGC	AAT	ACA	CAC	CTC	TGT	ATT	TCC	AAT	GGT	AAT	TAT	GAA	AAA	GAA	912
Ser	Gly	Asn	Thr	His	Leu	Cys	Ile	Ser	Asn	Gly	Asn	Tyr	Glu	Lys	Glu	
			930						935					940		

60

GGT CTC GGT GCT TCC AGC CAC ATA ACC ACA AAA TCA ATG GCT GCT CCT 960  
 Gly Leu Gly Ala Ser Ser His Ile Thr Thr Lys Ser Met Ala Ala Pro  
                   945                                  950                                  955

CCA AGC TGT GGT CTG AGC CCA CTG CTG GTC CTG GTG GTA ACC GCT CTG 1008  
 Pro Ser Cys Gly Leu Ser Pro Leu Leu Val Leu Val Val Thr Ala Leu  
                   960                                  965                                  970

TCC ACC CTA TTA TCT TTA ACA GAA ACA TCA TAGCTGCATT AAAAAAATAC 1058  
 Ser Thr Leu Leu Ser Leu Thr Glu Thr Ser  
                   975                                  980

AATATGGACA TGTA AAAAGA CAAAAACCAA GTTATCTGTT TCCTGTTCTC TTGTATAGCT 1118

GAAATTCAG TTTAGGAGCT CAGTTGAGAA ACAGTTCCAT TCAACTGGAA CATT TTTT TTTT 1178

TTTTCCTTTT AAGAAAGCTT CTTGTGATCC TTCGGGGCTT CTGTG 1223

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 346 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Leu Leu Glu Asp Ser Pro Tyr Glu Pro Val Asn Ser Arg Leu Ser Asp  
   1                                  5                                  10                                  15

Ile Phe Arg Val Val Pro Phe Ile Ser Val Glu His Ile Pro Lys Gly  
                                   20                                  25                                  30

Asn Asn Cys Leu Asp Ala Ala Lys Ala Cys Asn Leu Asp Asp Ile Cys  
                   35                                  40                                  45

Lys Lys Tyr Arg Ser Ala Tyr Ile Thr Pro Cys Thr Thr Ser Val Ser  
           50                                  55                                  60

Asn Asp Val Cys Asn Arg Arg Lys Cys His Lys Ala Leu Arg Gln Phe  
   65                                  70                                  75                                  80

Phe Asp Lys Val Pro Ala Lys His Ser Tyr Gly Met Leu Phe Cys Ser  
                                   85                                  90                                  95

Cys Arg Asp Ile Ala Cys Thr Glu Arg Arg Arg Gln Thr Ile Val Pro  
                   100                                  105                                  110

Val Cys Ser Tyr Glu Glu Arg Glu Lys Pro Asn Cys Leu Asn Leu Gln  
           115                                  120                                  125

C/

Asp Ser Cys Lys Thr Asn Tyr Ile Cys Arg Ser Arg Leu Ala Asp Phe  
 130 135 140  
 Phe Thr Asn Cys Gln Pro Glu Ser Arg Ser Val Ser Ser Cys Leu Lys  
 145 150 155 160  
 Glu Asn Tyr Ala Asp Cys Leu Leu Ala Tyr Ser Gly Leu Ile Gly Thr  
 165 170 175  
~~Val Met Thr Pro Asn Tyr Ile Asp Ser Ser Ser Leu Ser Val Ala Pro~~  
 180 185 190  
 Trp Cys Asp Cys Ser Asn Ser Gly Asn Asp Leu Glu Glu Cys Leu Lys  
 195 200 205  
 Phe Leu Asn Phe Phe Lys Asp Asn Thr Cys Leu Lys Asn Ala Ile Gln  
 210 215 220  
 Ala Phe Gly Asn Gly Ser Asp Val Thr Val Trp Gln Pro Ala Phe Pro  
 225 230 235 240  
 Val Gln Thr Thr Thr Ala Thr Thr Thr Thr Ala Leu Arg Val Lys Asn  
 245 250 255  
 Lys Pro Leu Gly Pro Ala Gly Ser Glu Asn Glu Ile Pro Thr His Val  
 260 265 270  
 Leu Pro Pro Cys Ala Asn Leu Gln Ala Gln Lys Leu Lys Ser Asn Val  
 275 280 285  
 Ser Gly Asn Thr His Leu Cys Ile Ser Asn Gly Asn Tyr Glu Lys Glu  
 290 295 300  
 Gly Leu Gly Ala Ser Ser His Ile Thr Thr Lys Ser Met Ala Ala Pro  
 305 310 315 320  
 Pro Ser Cys Gly Leu Ser Pro Leu Leu Val Leu Val Val Thr Ala Leu  
 325 330 335  
 Ser Thr Leu Leu Ser Leu Thr Glu Thr Ser  
 340 345

## (2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1682 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS

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(B) LOCATION: 118..1497

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGGCGGCCAG AGCAGCACAG CTGTCCGGGG ATCGCTGCAT GCTGAGCTCC CTCGGCAAGA	60
CCCAGCGGCG GCTCGGGATT TTTTGGGGG GCGGGGACC AGCCCCGCGC CGGCACC	117
ATG TTC CTG GCG ACC CTG TAC TTC GCG CTG CCG CTC TTG GAC TTG CTC Met Phe Leu Ala Thr Leu Tyr Phe Ala Leu Pro Leu Leu Asp Leu Leu	165
350 355 360	
GTG TCG GCC GAA GTG AGC GGC GGA GAC CGC CTG GAT TGC GTG AAA GCC Leu Ser Ala Glu Val Ser Gly Gly Asp Arg Leu Asp Cys Val Lys Ala	213
365 370 375	
AGT GAT CAG TGC CTG AAG GAG CAG AGC TGC AGC ACC AAG TAC CGC ACG Ser Asp Gln Cys Leu Lys Glu Gln Ser Cys Ser Thr Lys Tyr Arg Thr	261
380 385 390	
CTA AGG CAG TGC GTG GCG GGC AAG GAG ACC AAC TTC AGC CTG GCA TCC Leu Arg Gln Cys Val Ala Gly Lys Glu Thr Asn Phe Ser Leu Ala Ser	309
395 400 405 410	
GGC CTG GAG GCC AAG GAT GAG TGC CGC AGC GCC ATG GAG GCC CTG AAG Gly Leu Glu Ala Lys Asp Glu Cys Arg Ser Ala Met Glu Ala Leu Lys	357
415 420 425	
CAG AAG TCG CTC TAC AAC TGC CGC TGC AAG CGG GGT ATG AAG AAG GAG Gln Lys Ser Leu Tyr Asn Cys Arg Cys Lys Arg Gly Met Lys Lys Glu	405
430 435 440	
AAG AAC TGC CTG CGC ATT TAC TGG AGC ATG TAC CAG AGC CTG CAG GGA Lys Asn Cys Leu Arg Ile Tyr Trp Ser Met Tyr Gln Ser Leu Gln Gly	453
445 450 455	
AAT GAT CTG CTG GAG GAT TCC CCA TAT GAA CCA GTT AAC AGC AGA TTG Asn Asp Leu Leu Glu Asp Ser Pro Tyr Glu Pro Val Asn Ser Arg Leu	501
460 465 470	
TCA GAT ATA TTC CGG GTG GTC CCA TTC ATA TCA GTG GAG CAC ATT CCC Ser Asp Ile Phe Arg Val Val Pro Phe Ile Ser Val Glu His Ile Pro	549
475 480 485 490	
AAA GGG AAC AAC TGC CTG GAT GCA GCG AAG GCC TGC AAC CTC GAC GAC Lys Gly Asn Asn Cys Leu Asp Ala Ala Lys Ala Cys Asn Leu Asp Asp	597
495 500 505	
ATT TGC AAG AAG TAC AGG TCG GCG TAC ATC ACC CCG TGC ACC ACC AGC Ile Cys Lys Lys Tyr Arg Ser Ala Tyr Ile Thr Pro Cys Thr Thr Ser	645
510 515 520	

63

GTG	TCC	AAC	GAT	GTC	TGC	AAC	CGC	CGC	AAG	TGC	CAC	AAG	GCC	CTC	CGG	693
Val	Ser	Asn	Asp	Val	Cys	Asn	Arg	Arg	Lys	Cys	His	Lys	Ala	Leu	Arg	
		525					530					535				
CAG	TTC	TTT	GAC	AAG	GTC	CCG	GCC	AAG	CAC	AGC	TAC	GGA	ATG	CTC	TTC	741
Gln	Phe	Phe	Asp	Lys	Val	Pro	Ala	Lys	His	Ser	Tyr	Gly	Met	Leu	Phe	
		540					545				550					
TGC	TCC	TGC	CGG	GAC	ATC	GCC	TGC	ACA	GAG	CGG	AGG	CGA	CAG	ACC	ATC	789
Cys	Ser	Cys	Arg	Asp	Ile	Ala	Cys	Thr	Glu	Arg	Arg	Arg	Gln	Thr	Ile	
		555			560					565					570	
GTG	CCT	GTG	TGC	TCC	TAT	GAA	GAG	AGG	GAG	AAG	CCC	AAC	TGT	TTG	AAT	837
Val	Pro	Val	Cys	Ser	Tyr	Glu	Glu	Arg	Glu	Lys	Pro	Asn	Cys	Leu	Asn	
				575					580					585		
TTG	CAG	GAC	TCC	TGC	AAG	ACG	AAT	TAC	ATC	TGC	AGA	TCT	CGC	CTT	GCG	885
Leu	Gln	Asp	Ser	Cys	Lys	Thr	Asn	Tyr	Ile	Cys	Arg	Ser	Arg	Leu	Ala	
			590					595						600		
GAT	TTT	TTT	ACC	AAC	TGC	CAG	CCA	GAG	TCA	AGG	TCT	GTC	AGC	AGC	TGT	933
Asp	Phe	Phe	Thr	Asn	Cys	Gln	Pro	Glu	Ser	Arg	Ser	Val	Ser	Ser	Cys	
		605					610					615				
CTA	AAG	GAA	AAC	TAC	GCT	GAC	TGC	CTC	CTC	GCC	TAC	TCG	GGG	CTT	ATT	981
Leu	Lys	Glu	Asn	Tyr	Ala	Asp	Cys	Leu	Leu	Ala	Tyr	Ser	Gly	Leu	Ile	
		620				625					630					
GGC	ACA	GTC	ATG	ACC	CCC	AAC	TAC	ATA	GAC	TCC	AGT	AGC	CTC	AGT	GTG	1029
Gly	Thr	Val	Met	Thr	Pro	Asn	Tyr	Ile	Asp	Ser	Ser	Ser	Leu	Ser	Val	
		635				640				645					650	
GCC	CCA	TGG	TGT	GAC	TGC	AGC	AAC	AGT	GGG	AAC	GAC	CTA	GAA	GAG	TGC	1077
Ala	Pro	Trp	Cys	Asp	Cys	Ser	Asn	Ser	Gly	Asn	Asp	Leu	Glu	Glu	Cys	
				655					660					665		
TTG	AAA	TTT	TTG	AAT	TTC	TTC	AAG	GAC	AAT	ACA	TGT	CTT	AAA	AAT	GCA	1125
Leu	Lys	Phe	Leu	Asn	Phe	Phe	Lys	Asp	Asn	Thr	Cys	Leu	Lys	Asn	Ala	
			670					675					680			
ATT	CAA	GCC	TTT	GGC	AAT	GGC	TCC	GAT	GTG	ACC	GTG	TGG	CAG	CCA	GCC	1173
Ile	Gln	Ala	Phe	Gly	Asn	Gly	Ser	Asp	Val	Thr	Val	Trp	Gln	Pro	Ala	
		685					690					695				
TTC	CCA	GTA	CAG	ACC	ACC	ACT	GCC	ACT	ACC	ACC	ACT	GCC	CTC	CGG	GTT	1221
Phe	Pro	Val	Gln	Thr	Thr	Thr	Ala	Thr	Thr	Thr	Thr	Thr	Ala	Leu	Arg	
		700					705					710				
AAG	AAC	AAG	CCC	CTG	GGG	CCA	GCA	GGG	TCT	GAG	AAT	GAA	ATT	CCC	ACT	1269
Lys	Asn	Lys	Pro	Leu	Gly	Pro	Ala	Gly	Ser	Glu	Asn	Glu	Ile	Pro	Thr	
		715			720					725				730		
CAT	GTT	TTG	CCA	CCG	TGT	GCA	AAT	TTA	CAG	GCA	CAG	AAG	CTG	AAA	TCC	1317
His	Val	Leu	Pro	Pro	Cys	Ala	Asn	Leu	Gln	Ala	Gln	Lys	Leu	Lys	Ser	
				735					740					745		

64

AAT GTG TCG GGC AAT ACA CAC CTC TGT ATT TCC AAT GGT AAT TAT GAA	1365
Asn Val Ser Gly Asn Thr His Leu Cys Ile Ser Asn Gly Asn Tyr Glu	
750 755 760	
AAA GAA GGT CTC GGT GCT TCC AGC CAC ATA ACC ACA AAA TCA ATG GCT	1413
Lys Glu Gly Leu Gly Ala Ser Ser His Ile Thr Thr Lys Ser Met Ala	
765 770 775	
GCT CCT CCA AGC TGT GGT CTG AGC CCA CTG CTG GTC CTG GTG GTA ACC	1461
Ala Pro Pro Ser Cys Gly Leu Ser Pro Leu Leu Val Leu Val Val Thr	
780 785 790	
GCT CTG TCC ACC CTA TTA TCT TTA ACA GAA ACA TCA TAGCTGCATT	1507
Ala Leu Ser Thr Leu Leu Ser Leu Thr Glu Thr Ser	
795 800 805	
AAAAAAATAC AATATGGACA TGTA AAAAGA CAAAACCAA GTTATCTGTT TCCTGTTCTC	1567
TTGTATAGCT GAAATTCCAG TTTAGGAGCT CAGTTGAGAA ACAGTTCCAT TCAACTGGAA	1627
CATTTTTTTTT TTTTCCTTTT AAGAAAGCTT CTTGTGATCC TTCGGGGCTT CTGTG	1682

## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 460 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met	Phe	Leu	Ala	Thr	Leu	Tyr	Phe	Ala	Leu	Pro	Leu	Leu	Asp	Leu	Leu
1				5					10				15		
Leu	Ser	Ala	Glu	Val	Ser	Gly	Gly	Asp	Arg	Leu	Asp	Cys	Val	Lys	Ala
			20					25					30		
Ser	Asp	Gln	Cys	Leu	Lys	Glu	Gln	Ser	Cys	Ser	Thr	Lys	Tyr	Arg	Thr
		35					40					45			
Leu	Arg	Gln	Cys	Val	Ala	Gly	Lys	Glu	Thr	Asn	Phe	Ser	Leu	Ala	Ser
	50					55					60				
Gly	Leu	Glu	Ala	Lys	Asp	Glu	Cys	Arg	Ser	Ala	Met	Glu	Ala	Leu	Lys
65				70					75					80	
Gln	Lys	Ser	Leu	Tyr	Asn	Cys	Arg	Cys	Lys	Arg	Gly	Met	Lys	Lys	Glu
			85						90					95	
Lys	Asn	Cys	Leu	Arg	Ile	Tyr	Trp	Ser	Met	Tyr	Gln	Ser	Leu	Gln	Gly
			100					105					110		

Asn Asp Leu Leu Glu Asp Ser Pro Tyr Glu Pro Val Asn Ser Arg Leu  
 115 120 125  
 Ser Asp Ile Phe Arg Val Val Pro Phe Ile Ser Val Glu His Ile Pro  
 130 135 140  
 Lys Gly Asn Asn Cys Leu Asp Ala Ala Lys Ala Cys Asn Leu Asp Asp  
 145 150 155 160  
 Ile Cys Lys Lys Tyr Arg Ser Ala Tyr Ile Thr Pro Cys Thr Thr Ser  
 165 170 175  
 Val Ser Asn Asp Val Cys Asn Arg Arg Lys Cys His Lys Ala Leu Arg  
 180 185 190  
 Gln Phe Phe Asp Lys Val Pro Ala Lys His Ser Tyr Gly Met Leu Phe  
 195 200 205  
 Cys Ser Cys Arg Asp Ile Ala Cys Thr Glu Arg Arg Arg Gln Thr Ile  
 210 215 220  
 Val Pro Val Cys Ser Tyr Glu Glu Arg Glu Lys Pro Asn Cys Leu Asn  
 225 230 235 240  
 Leu Gln Asp Ser Cys Lys Thr Asn Tyr Ile Cys Arg Ser Arg Leu Ala  
 245 250 255  
 Asp Phe Phe Thr Asn Cys Gln Pro Glu Ser Arg Ser Val Ser Ser Cys  
 260 265 270  
 Leu Lys Glu Asn Tyr Ala Asp Cys Leu Leu Ala Tyr Ser Gly Leu Ile  
 275 280 285  
 Gly Thr Val Met Thr Pro Asn Tyr Ile Asp Ser Ser Ser Leu Ser Val  
 290 295 300  
 Ala Pro Trp Cys Asp Cys Ser Asn Ser Gly Asn Asp Leu Glu Glu Cys  
 305 310 315 320  
 Leu Lys Phe Leu Asn Phe Phe Lys Asp Asn Thr Cys Leu Lys Asn Ala  
 325 330 335  
 Ile Gln Ala Phe Gly Asn Gly Ser Asp Val Thr Val Trp Gln Pro Ala  
 340 345 350  
 Phe Pro Val Gln Thr Thr Thr Ala Thr Thr Thr Thr Ala Leu Arg Val  
 355 360 365  
 Lys Asn Lys Pro Leu Gly Pro Ala Gly Ser Glu Asn Glu Ile Pro Thr  
 370 375 380  
 His Val Leu Pro Pro Cys Ala Asn Leu Gln Ala Gln Lys Leu Lys Ser  
 385 390 395 400

Asn Val Ser Gly Asn Thr His Leu Cys Ile Ser Asn Gly Asn Tyr Glu  
405 410 415

Lys Glu Gly Leu Gly Ala Ser Ser His Ile Thr Thr Lys Ser Met Ala  
420 425 430

Ala Pro Pro Ser Cys Gly Leu Ser Pro Leu Leu Val Leu Val Val Thr  
435 440 445

Ala Leu Ser Thr Leu Leu Ser Leu Thr Glu Thr Ser  
450 455 460

## (2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1888 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) **FEATURE:**

- (A) NAME/KEY: CDS  
(B) LOCATION: 25..1416

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AAAAAACGGT GGGATTTATT TAAC ATG ATC TTG GCA AAC GTC TTC TGC CTC 51  
Met Ile Leu Ala Asn Val Phe Cys Leu  
465

TTC	TTC	TTT	CTA	GAC	GAG	ACC	CTC	CGC	TCT	TTG	GCC	AGC	CCT	TCC	TCC	99
Phe	Phe	Phe	Leu	Asp	Glu	Thr	Leu	Arg	Ser	Leu	Ala	Ser	Pro	Ser	Ser	
470					475					480					485	

CTG	CAG	GGC	CCC	GAG	CTC	CAC	GGC	TGG	CGC	CCC	CCA	GTG	GAC	TGT	GTC	147
Leu	Gln	Gly	Pro	Glu	Leu	His	Gly	Trp	Arg	Pro	Pro	Val	Asp	Cys	Val	
				490					495					500		

CGG GCC AAT GAG CTG TGT GCC GCC GAA TCC AAC TGC AGC TCT CGC TAC 195  
Arg Ala Asn Glu Leu Cys Ala Ala Glu Ser Asn Cys Ser Ser Arg Tyr  
505 510 515

CGC ACT CTG CGG CAG TGC CTG GCA GGC CGC GAC CGC AAC ACC ATG CTG 243  
Arg Thr Leu Arg Gln Cys Leu Ala Gly Arg Asp Arg Asn Thr Met Leu  
520 525 530

GCC AAC AAG GAG TGC CAG GCG GCC TTG GAG GTC TTG CAG GAG AGC CCG 291  
Ala Asn Lys Glu Cys Gln Ala Ala Leu Glu Val Leu Gln Glu Ser Pro  
535 540 545



CTG	TAC	GAC	TGC	CGC	TGC	AAG	CGG	GGC	ATG	AAG	AAG	GAG	CTG	CAG	TGT	339
Leu	Tyr	Asp	Cys	Arg	Cys	Lys	Arg	Gly	Met	Lys	Lys	Glu	Leu	Gln	Cys	
550					555					560					565	
CTG	CAG	ATC	TAC	TGG	AGC	ATC	CAC	CTG	GGG	CTG	ACC	GAG	GGT	GAG	GAG	387
Leu	Gln	Ile	Tyr	Trp	Ser	Ile	His	Leu	Gly	Leu	Thr	Glu	Gly	Glu	Glu	
				570					575					580		
TTC	TAC	GAA	GCC	TCC	CCC	TAT	GAG	CCG	GTG	ACC	TCC	CGC	CTC	TCG	GAC	435
Phe	Tyr	Glu	Ala	Ser	Pro	Tyr	Glu	Pro	Val	Thr	Ser	Arg	Leu	Ser	Asp	
			585					590					595			
ATC	TTC	AGG	CTT	GCT	TCA	ATC	TTC	TCA	GGG	ACA	GGG	GCA	GAC	CCG	GTG	483
Ile	Phe	Arg	Leu	Ala	Ser	Ile	Phe	Ser	Gly	Thr	Gly	Ala	Asp	Pro	Val	
		600					605					610				
GTC	AGC	GCC	AAG	AGC	AAC	CAT	TGC	CTG	GAT	GCT	GCC	AAG	GCC	TGC	AAC	531
Val	Ser	Ala	Lys	Ser	Asn	His	Cys	Leu	Asp	Ala	Ala	Lys	Ala	Cys	Asn	
	615					620					625					
CTG	AAT	GAC	AAC	TGC	AAG	AAG	CTG	CGC	TCC	TCC	TAC	ATC	TCC	ATC	TGC	579
Leu	Asn	Asp	Asn	Cys	Lys	Lys	Leu	Arg	Ser	Ser	Tyr	Ile	Ser	Ile	Cys	
630					635					640					645	
AAC	CGC	GAG	ATC	TCG	CCC	ACC	GAG	CGC	TGC	AAC	CGC	CGC	AAG	TGC	CAC	627
Asn	Arg	Glu	Ile	Ser	Pro	Thr	Glu	Arg	Cys	Asn	Arg	Arg	Lys	Cys	His	
				650					655					660		
AAG	GCC	CTG	CGC	CAG	TTC	TTC	GAC	CGG	GTG	CCC	AGC	GAG	TAC	ACC	TAC	675
Lys	Ala	Leu	Arg	Gln	Phe	Phe	Asp	Arg	Val	Pro	Ser	Glu	Tyr	Thr	Tyr	
			665					670					675			
CGC	ATG	CTC	TTC	TGC	TCC	TGC	CAA	GAC	CAG	GCG	TGC	GCT	GAG	CGC	CGC	723
Arg	Met	Leu	Phe	Cys	Ser	Cys	Gln	Asp	Gln	Ala	Cys	Ala	Glu	Arg	Arg	
		680					685					690				
CGG	CAA	ACC	ATC	CTG	CCC	AGC	TGC	TCC	TAT	GAG	GAC	AAG	GAG	AAG	CCC	771
Arg	Gln	Thr	Ile	Leu	Pro	Ser	Cys	Ser	Tyr	Glu	Asp	Lys	Glu	Lys	Pro	
	695					700					705					
AAC	TGC	CTG	GAC	CTG	CGT	GGC	GTG	TGC	CGG	ACT	GAC	CAC	CTG	TGT	CGG	819
Asn	Cys	Leu	Asp	Leu	Arg	Gly	Val	Cys	Arg	Thr	Asp	His	Leu	Cys	Arg	
710					715					720					725	
TCC	CGG	CTG	GCC	GAC	TTC	CAT	GCC	AAT	TGT	CGA	GCC	TCC	TAC	CAG	ACG	867
Ser	Arg	Leu	Ala	Asp	Phe	His	Ala	Asn	Cys	Arg	Ala	Ser	Tyr	Gln	Thr	
				730					735					740		
GTC	ACC	AGC	TGC	CCT	GCG	GAC	AAT	TAC	CAG	GCG	TGT	CTG	GGC	TCT	TAT	915
Val	Thr	Ser	Cys	Pro	Ala	Asp	Asn	Tyr	Gln	Ala	Cys	Leu	Gly	Ser	Tyr	
			745					750					755			
GCT	GGC	ATG	ATT	GGG	TTT	GAC	ATG	ACA	CCT	AAC	TAT	GTG	GAC	TCC	AGC	963
Ala	Gly	Met	Ile	Gly	Phe	Asp	Met	Thr	Pro	Asn	Tyr	Val	Asp	Ser	Ser	
		760					765					770				

CCC ACT GGC ATC GTG GTG TCC CCC TGG TGC AGC TGT CGT GGC AGC GGG Pro Thr Gly Ile Val Val Ser Pro Trp Cys Ser Cys Arg Gly Ser Gly 775 780 785	1011
AAC ATG GAG GAG GAG TGT GAG AAG TTC CTC AGG GAC TTC ACC GAG AAC Asn Met Glu Glu Glu Cys Glu Lys Phe Leu Arg Asp Phe Thr Glu Asn 790 795 800 805	1059
CCA TGC CTC CGG AAC GCC ATC CAG GCC TTT GGC AAC GGC ACG GAC GTG Pro Cys Leu Arg Asn Ala Ile Gln Ala Phe Gly Asn Gly Thr Asp Val 810 815 820	1107
AAC GTG TCC CCA AAA GGC CCC TCG TTC CAG GCC ACC CAG GCC CCT CGG Asn Val Ser Pro Lys Gly Pro Ser Phe Gln Ala Thr Gln Ala Pro Arg 825 830 835	1155
GTG GAG AAG ACG CCT TCT TTG CCA GAT GAC CTC AGT GAC AGT ACC AGC Val Glu Lys Thr Pro Ser Leu Pro Asp Asp Leu Ser Asp Ser Thr Ser 840 845 850	1203
TTG GGG ACC AGT GTC ATC ACC ACC TGC ACG TCT GTC CAG GAG CAG GGG Leu Gly Thr Ser Val Ile Thr Thr Cys Thr Ser Val Gln Glu Gln Gly 855 860 865	1251
CTG AAG GCC AAC AAC TCC AAA GAG TTA AGC ATG TGC TTC ACA GAG CTC Leu Lys Ala Asn Asn Ser Lys Glu Leu Ser Met Cys Phe Thr Glu Leu 870 875 880 885	1299
ACG ACA AAT ATC ATC CCA GGG AGT AAC AAG GTG ATC AAA CCT AAC TCA Thr Thr Asn Ile Ile Pro Gly Ser Asn Lys Val Ile Lys Pro Asn Ser 890 895 900	1347
GGC CCC AGC AGA GCC AGA CCG TCG GCT GCC TTG ACC GTG CTG TCT GTC Gly Pro Ser Arg Ala Arg Pro Ser Ala Ala Leu Thr Val Leu Ser Val 905 910 915	1395
CTG ATG CTG AAA CTG GCC TTG TAGGCTGTGG GAACCGAGTC AGAAGATTTT Leu Met Leu Lys Leu Ala Leu 920	1446
TGAAAGCTAC GCAGACAAGA ACAGCCGCCT GACGAAATGG AAACACACAC AGACACACAC	1506
ACACCTTGCA AAAAAAAAAAT TGTTTTTCCC ACCTTGTCGC TGAACCTGTC TCCTCCCAGG	1566
TTTCTTCTCT GGAGAAGTTT TTGTAAACCA AACAGACAAG CAGGCAGGCA GCCTGAGAGC	1626
TGGCCCAGGG GTCCCCTGGC AGGGGAAACT CTGGTGCCGG GGAGGGCACG AGGCTCTAGA	1686
AATGCCCTTC ACTTTCTCCT GGTGTTTTTC TCTCTGGACC CTTCTGAAGC AGAGACCGGA	1746
CAAGAGCCTG CAGCGGAAGG GACTCTGGGC TGTGCCTGAG GCTGGCTGGG GGCAGGACAA	1806
CACAGCTGCT TCCCCAGGCT GCCCACTCTG GGGACCCGCT GGGGGCTGGC AGAGGGCATC	1866

GGTCAGCGGG GCAGCGGGGC TG

1888

## (2) INFORMATION FOR SEQ ID NO:13:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 464 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met	Ile	Leu	Ala	Asn	Val	Phe	Cys	Leu	Phe	Phe	Phe	Leu	Asp	Glu	Thr	1	5	10	15
Leu	Arg	Ser	Leu	Ala	Ser	Pro	Ser	Ser	Leu	Gln	Gly	Pro	Glu	Leu	His	20	25	30	
Gly	Trp	Arg	Pro	Pro	Val	Asp	Cys	Val	Arg	Ala	Asn	Glu	Leu	Cys	Ala	35	40	45	
Ala	Glu	Ser	Asn	Cys	Ser	Ser	Arg	Tyr	Arg	Thr	Leu	Arg	Gln	Cys	Leu	50	55	60	
Ala	Gly	Arg	Asp	Arg	Asn	Thr	Met	Leu	Ala	Asn	Lys	Glu	Cys	Gln	Ala	65	70	75	80
Ala	Leu	Glu	Val	Leu	Gln	Glu	Ser	Pro	Leu	Tyr	Asp	Cys	Arg	Cys	Lys	85	90	95	
Arg	Gly	Met	Lys	Lys	Glu	Leu	Gln	Cys	Leu	Gln	Ile	Tyr	Trp	Ser	Ile	100	105	110	
His	Leu	Gly	Leu	Thr	Glu	Gly	Glu	Glu	Phe	Tyr	Glu	Ala	Ser	Pro	Tyr	115	120	125	
Glu	Pro	Val	Thr	Ser	Arg	Leu	Ser	Asp	Ile	Phe	Arg	Leu	Ala	Ser	Ile	130	135	140	
Phe	Ser	Gly	Thr	Gly	Ala	Asp	Pro	Val	Val	Ser	Ala	Lys	Ser	Asn	His	145	150	155	160
Cys	Leu	Asp	Ala	Ala	Lys	Ala	Cys	Asn	Leu	Asn	Asp	Asn	Cys	Lys	Lys	165	170	175	
Leu	Arg	Ser	Ser	Tyr	Ile	Ser	Ile	Cys	Asn	Arg	Glu	Ile	Ser	Pro	Thr	180	185	190	
Glu	Arg	Cys	Asn	Arg	Arg	Lys	Cys	His	Lys	Ala	Leu	Arg	Gln	Phe	Phe	195	200	205	
Asp	Arg	Val	Pro	Ser	Glu	Tyr	Thr	Tyr	Arg	Met	Leu	Phe	Cys	Ser	Cys	210	215	220	

Gln Asp Gln Ala Cys Ala Glu Arg Arg Arg Gln Thr Ile Leu Pro Ser  
 225 230 235 240  
 Cys Ser Tyr Glu Asp Lys Glu Lys Pro Asn Cys Leu Asp Leu Arg Gly  
 245 250 255  
 Val Cys Arg Thr Asp His Leu Cys Arg Ser Arg Leu Ala Asp Phe His  
 260 265 270  
 Ala Asn Cys Arg Ala Ser Tyr Gln Thr Val Thr Ser Cys Pro Ala Asp  
 275 280 285  
 Asn Tyr Gln Ala Cys Leu Gly Ser Tyr Ala Gly Met Ile Gly Phe Asp  
 290 295 300  
 Met Thr Pro Asn Tyr Val Asp Ser Ser Pro Thr Gly Ile Val Val Ser  
 305 310 315 320  
 Pro Trp Cys Ser Cys Arg Gly Ser Gly Asn Met Glu Glu Glu Cys Glu  
 325 330 335  
 Lys Phe Leu Arg Asp Phe Thr Glu Asn Pro Cys Leu Arg Asn Ala Ile  
 340 345 350  
 Gln Ala Phe Gly Asn Gly Thr Asp Val Asn Val Ser Pro Lys Gly Pro  
 355 360 365  
 Ser Phe Gln Ala Thr Gln Ala Pro Arg Val Glu Lys Thr Pro Ser Leu  
 370 375 380  
 Pro Asp Asp Leu Ser Asp Ser Thr Ser Leu Gly Thr Ser Val Ile Thr  
 385 390 395 400  
 Thr Cys Thr Ser Val Gln Glu Gln Gly Leu Lys Ala Asn Asn Ser Lys  
 405 410 415  
 Glu Leu Ser Met Cys Phe Thr Glu Leu Thr Thr Asn Ile Ile Pro Gly  
 420 425 430  
 Ser Asn Lys Val Ile Lys Pro Asn Ser Gly Pro Ser Arg Ala Arg Pro  
 435 440 445  
 Ser Ala Ala Leu Thr Val Leu Ser Val Leu Met Leu Lys Leu Ala Leu  
 450 455 460

## (2) INFORMATION FOR SEQ ID NO:14:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1878 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 205..1242

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CGCGGCGCCC AGCGCAGGCA GAGCGCTGTC GCATCCCGGG CGTCCACCCG CCATGGGGCT	60
CTCCTGGAGC CCGCGACCTC CACTGCTGAT GATCCTGCTA CTGGTGCTGT CGTTGTGGCT	120
GCCACTTGGA GCAGGAACT CCCTTGCCAC AGAGAACAGG TTTGTGAACA GCTGTACCCA	180
GGCCAGAAAG AAATGCGAGG CTAA TCC CGC TTG CAA GGC TGC CTA CCA GCA	231
Ser Arg Leu Gln Gly Cys Leu Pro Ala	
465 470	
CCT GGG CTC CTG CAC CTC CAG TTA AGC AGG CCG CTG CCC TTA GAG GAG	279
Pro Gly Leu Leu His Leu Gln Leu Ser Arg Pro Leu Pro Leu Glu Glu	
475 480 485	
TCT GCC ATG TCT GCA GAC TGC CTA GAG GCA GCA GAA CAA CTC AGG AAC	327
Ser Ala Met Ser Ala Asp Cys Leu Glu Ala Ala Glu Gln Leu Arg Asn	
490 495 500 505	
AGC TCT CTG ATA GAC TGC AGG TGC CAT CGG CGC ATG AAG CAC CAA GCT	375
Ser Ser Leu Ile Asp Cys Arg Cys His Arg Arg Met Lys His Gln Ala	
510 515 520	
ACC TGT CTG GAC ATT TAT TGG ACC GTT CAC CCT GCC CGA AGC CTT GGT	423
Thr Cys Leu Asp Ile Tyr Trp Thr Val His Pro Ala Arg Ser Leu Gly	
525 530 535	
GAC TAC GAG TTG GAT GTC TCA CCC TAT GAA GAC ACA GTG ACC AGC AAA	471
Asp Tyr Glu Leu Asp Val Ser Pro Tyr Glu Asp Thr Val Thr Ser Lys	
540 545 550	
CCC TGG AAA ATG AAT CTT AGC AAG TTG AAC ATG CTC AAA CCA GAC TCG	519
Pro Trp Lys Met Asn Leu Ser Lys Leu Asn Met Leu Lys Pro Asp Ser	
555 560 565	
GAC CTC TGC CTC AAA TTT GCT ATG CTG TGT ACT CTT CAC GAC AAG TGT	567
Asp Leu Cys Leu Lys Phe Ala Met Leu Cys Thr Leu His Asp Lys Cys	
570 575 580 585	
GAC CGC CTG CGC AAG GCC TAC GGG GAG GCA TGC TCA GGG ATC CGC TGC	615
Asp Arg Leu Arg Lys Ala Tyr Gly Glu Ala Cys Ser Gly Ile Arg Cys	
590 595 600	
CAG CGC CAC CTC TGC CTA GCC CAG CTG CGC TCC TTC TTT GAG AAG GCA	663
Gln Arg His Leu Cys Leu Ala Gln Leu Arg Ser Phe Phe Glu Lys Ala	
605 610 615	

GCA GAG TCC CAC GCT CAG GGT CTG CTG CTG TGT CCC TGT GCA CCA GAA Ala Glu Ser His Ala Gln Gly Leu Leu Leu Cys Pro Cys Ala Pro Glu 620 625 630	711
GAT GCG GGC TGT GGG GAG CGG CGG CGT AAC ACC ATC GCC CCC AGT TGC Asp Ala Gly Cys Gly Glu Arg Arg Arg Asn Thr Ile Ala Pro Ser Cys 635 640 645	759
GCC CTG CCT TCT GTA ACC CCC AAT TGC CTG GAT CTG CGG AGC TTC TGC Ala Leu Pro Ser Val Thr Pro Asn Cys Leu Asp Leu Arg Ser Phe Cys 650 655 660 665	807
CGT GCG GAC CCT TTG TGC AGA TCA CGC CTG ATG GAC TTC CAG ACC CAC Arg Ala Asp Pro Leu Cys Arg Ser Arg Leu Met Asp Phe Gln Thr His 670 675 680	855
TGT CAT CCT ATG GAC ATC CTT GGG ACT TGT GCA ACT GAG CAG TCC AGA Cys His Pro Met Asp Ile Leu Gly Thr Cys Ala Thr Glu Gln Ser Arg 685 690 695	903
TGT CTG CGG GCA TAC CTG GGG CTG ATT GGG ACT GCC ATG ACC CCA AAC Cys Leu Arg Ala Tyr Leu Gly Leu Ile Gly Thr Ala Met Thr Pro Asn 700 705 710	951
TTC ATC AGC AAG GTC AAC ACT ACT GTT GCC TTA AGC TGC ACC TGC CGA Phe Ile Ser Lys Val Asn Thr Thr Val Ala Leu Ser Cys Thr Cys Arg 715 720 725	999
GGC AGC GGC AAC CTA CAG GAC GAG TGT GAA CAG CTG GAA AGG TCC TTC Gly Ser Gly Asn Leu Gln Asp Glu Cys Glu Gln Leu Glu Arg Ser Phe 730 735 740 745	1047
TCC CAG AAC CCC TGC CTC GTG GAG GCC ATT GCA GCT AAG ATG CGT TTC Ser Gln Asn Pro Cys Leu Val Glu Ala Ile Ala Ala Lys Met Arg Phe 750 755 760	1095
CAC AGA CAG CTC TTC TCC CAG GAC TGG GCA GAC TCT ACT TTT TCA GTG His Arg Gln Leu Phe Ser Gln Asp Trp Ala Asp Ser Thr Phe Ser Val 765 770 775	1143
GTG CAG CAG CAG AAC AGC AAC CCT GCT CTG AGA CTG CAG CCC AGG CTA Val Gln Gln Gln Asn Ser Asn Pro Ala Leu Arg Leu Gln Pro Arg Leu 780 785 790	1191
CCC ATT CTT TCT TTC TCC ATC CTT CCC TTG ATT CTG CTG CAG ACC CTC Pro Ile Leu Ser Phe Ser Ile Leu Pro Leu Ile Leu Leu Gln Thr Leu 795 800 805	1239
TGG TAGCTGGGCT TCCTCAGGGT CCTTTGTCCT CTCCACCACA CCCAGACTGA Trp 810	1292
TTTGCAACCT GTGGTGGGAG AGAACTCGCC AGCCTGTGGA AGAAGACGCA GCGTGCTACA	1352

CAGCAACCCG GAACCAACCA GGCATTCCGC AGCACATCCC GTCTGCTCCA GAAGAGGTCT 1412  
 TAGAAGTGAG GGCTGTGACC CTTCCGATCC TGAGCGGCTA GTTTTCAAAC CTCCCTTGCC 1472  
 CCTGCTTCCT TCTGGCTCAG GCTGCTCCTC CTTAGGACTT TGTGGGTCCA GTTTTGCCTT 1532  
 CTGTTCTGAT GGTGATTAGC GGCTCACCTC CAGCGCTTCT TCCTGTTTCC CAGGACCACC 1592  
 CAGAGGCTAA GGAATCAGTC ATTCCCTGTT GCCTTCTCCA GGAAGGCAGG CTAAGGGTTC 1652  
 TGAGGTGACT GAGAAAAATG TTTCTTTTGT GTGGAAGGCT GGTGCTCCAG CCTCCACGTC 1712  
 CCTCTGAATG GAAGATAAAA ACCTGCTGGT GTCTTGACTG CTCTGCCAGG CAATCCTGAA 1772  
 CATTTGGGCA TGAAGAGCTA AAGTCTTTGG GTCTTGTTTA ACTCCTATTA CTGTCCCCAA 1832  
 ATTCCCCTAG TCCCTTGGGT CATGATTAAA CATTTTGACT TAAAAA 1878

## (2) INFORMATION FOR SEQ ID NO:15:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 346 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Ser Arg Leu Gln Gly Cys Leu Pro Ala Pro Gly Leu Leu His Leu Gln  
 1 5 10 15  
 Leu Ser Arg Pro Leu Pro Leu Glu Glu Ser Ala Met Ser Ala Asp Cys  
 20 25 30  
 Leu Glu Ala Ala Glu Gln Leu Arg Asn Ser Ser Leu Ile Asp Cys Arg  
 35 40 45  
 Cys His Arg Arg Met Lys His Gln Ala Thr Cys Leu Asp Ile Tyr Trp  
 50 55 60  
 Thr Val His Pro Ala Arg Ser Leu Gly Asp Tyr Glu Leu Asp Val Ser  
 65 70 75 80  
 Pro Tyr Glu Asp Thr Val Thr Ser Lys Pro Trp Lys Met Asn Leu Ser  
 85 90 95  
 Lys Leu Asn Met Leu Lys Pro Asp Ser Asp Leu Cys Leu Lys Phe Ala  
 100 105 110  
 Met Leu Cys Thr Leu His Asp Lys Cys Asp Arg Leu Arg Lys Ala Tyr  
 115 120 125

Gly Glu Ala Cys Ser Gly Ile Arg Cys Gln Arg His Leu Cys Leu Ala  
 130 135 140  
 Gln Leu Arg Ser Phe Phe Glu Lys Ala Ala Glu Ser His Ala Gln Gly  
 145 150 155 160  
 Leu Leu Leu Cys Pro Cys Ala Pro Glu Asp Ala Gly Cys Gly Glu Arg  
 165 170 175  
 Arg Arg Asn Thr Ile Ala Pro Ser Cys Ala Leu Pro Ser Val Thr Pro  
 180 185 190  
 Asn Cys Leu Asp Leu Arg Ser Phe Cys Arg Ala Asp Pro Leu Cys Arg  
 195 200 205  
 Ser Arg Leu Met Asp Phe Gln Thr His Cys His Pro Met Asp Ile Leu  
 210 215 220  
 Gly Thr Cys Ala Thr Glu Gln Ser Arg Cys Leu Arg Ala Tyr Leu Gly  
 225 230 235 240  
 Leu Ile Gly Thr Ala Met Thr Pro Asn Phe Ile Ser Lys Val Asn Thr  
 245 250 255  
 Thr Val Ala Leu Ser Cys Thr Cys Arg Gly Ser Gly Asn Leu Gln Asp  
 260 265 270  
 Glu Cys Glu Gln Leu Glu Arg Ser Phe Ser Gln Asn Pro Cys Leu Val  
 275 280 285  
 Glu Ala Ile Ala Ala Lys Met Arg Phe His Arg Gln Leu Phe Ser Gln  
 290 295 300  
 Asp Trp Ala Asp Ser Thr Phe Ser Val Val Gln Gln Gln Asn Ser Asn  
 305 310 315 320  
 Pro Ala Leu Arg Leu Gln Pro Arg Leu Pro Ile Leu Ser Phe Ser Ile  
 325 330 335  
 Leu Pro Leu Ile Leu Leu Gln Thr Leu Trp  
 340 345

## (2) INFORMATION FOR SEQ ID NO:16:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1889 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS



(B) LOCATION: 41..1231

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CGCAGGCAGA GCGCTGTCGC ATCCCGGGCG TCCACCCGCC	ATG GGG CTC TCC TGG	55
	Met Gly Leu Ser Trp	
	350	
AGC CCG CGA CCT CCA CTG CTG ATG ATC CTG CTA CTG GTG CTG TCG TTG		103
Ser Pro Arg Pro Pro Leu Leu Met Ile Leu Leu Leu Val Leu Ser Leu		
355 360 365		
TGG CTG CCA CTT GGA GCA GGA AAC TCC CTT GCC ACA GAG AAC AGG TTT		151
Trp Leu Pro Leu Gly Ala Gly Asn Ser Leu Ala Thr Glu Asn Arg Phe		
370 375 380		
GTG AAC AGC TGT ACC CAG GCC AGA AAG AAA TGC GAG GCT AAT CCC GCT		199
Val Asn Ser Cys Thr Gln Ala Arg Lys Lys Cys Glu Ala Asn Pro Ala		
385 390 395		
TGC AAG GCT GCC TAC CAG CAC CTG GGC TCC TGC ACC TCC AGT TTA AGC		247
Cys Lys Ala Ala Tyr Gln His Leu Gly Ser Cys Thr Ser Ser Leu Ser		
400 405 410 415		
AGG CCG CTG CCC TTA GAG GAG TCT GCC ATG TCT GCA GAC TGC CTA GAG		295
Arg Pro Leu Pro Leu Glu Glu Ser Ala Met Ser Ala Asp Cys Leu Glu		
420 425 430		
GCA GCA GAA CAA CTC AGG AAC AGC TCT CTG ATA GAC TGC AGG TGC CAT		343
Ala Ala Glu Gln Leu Arg Asn Ser Ser Leu Ile Asp Cys Arg Cys His		
435 440 445		
CGG CGC ATG AAG CAC CAA GCT ACC TGT CTG GAC ATT TAT TGG ACC GTT		391
Arg Arg Met Lys His Gln Ala Thr Cys Leu Asp Ile Tyr Trp Thr Val		
450 455 460		
CAC CCT GCC CGA AGC CTT GGT GAC TAC GAG TTG GAT GTC TCA CCC TAT		439
His Pro Ala Arg Ser Leu Gly Asp Tyr Glu Leu Asp Val Ser Pro Tyr		
465 470 475		
GAA GAC ACA GTG ACC AGC AAA CCC TGG AAA ATG AAT CTT AGC AAG TTG		487
Glu Asp Thr Val Thr Ser Lys Pro Trp Lys Met Asn Leu Ser Lys Leu		
480 485 490 495		
AAC ATG CTC AAA CCA GAC TCG GAC CTC TGC CTC AAA TTT GCT ATG CTG		535
Asn Met Leu Lys Pro Asp Ser Asp Leu Cys Leu Lys Phe Ala Met Leu		
500 505 510		
TGT ACT CTT CAC GAC AAG TGT GAC CGC CTG CGC AAG GCC TAC GGG GAG		583
Cys Thr Leu His Asp Lys Cys Asp Arg Leu Arg Lys Ala Tyr Gly Glu		
515 520 525		

GCA TGC TCA GGG ATC CGC TGC CAG CGC CAC CTC TGC CTA GCC CAG CTG Ala Cys Ser Gly Ile Arg Cys Gln Arg His Leu Cys Leu Ala Gln Leu 530 535 540	631
CGC TCC TTC TTT GAG AAG GCA GCA GAG TCC CAC GCT CAG GGT CTG CTG Arg Ser Phe Phe Glu Lys Ala Ala Glu Ser His Ala Gln Gly Leu Leu 545 550 555	679
CTG TGT CCC TGT GCA CCA GAA GAT GCG GGC TGT GGG GAG CGG CGG CGT Leu Cys Pro Cys Ala Pro Glu Asp Ala Gly Cys Gly Glu Arg Arg Arg 560 565 570 575	727
AAC ACC ATC GCC CCC AGT TGC GCC CTG CCT TCT GTA ACC CCC AAT TGC Asn-Thr Ile Ala Pro Ser Cys Ala Leu Pro Ser Val Thr Pro Asn Cys 580 585 590	775
CTG GAT CTG CGG AGC TTC TGC CGT GCG GAC CCT TTG TGC AGA TCA CGC Leu Asp Leu Arg Ser Phe Cys Arg Ala Asp Pro Leu Cys Arg Ser Arg 595 600 605	823
CTG ATG GAC TTC CAG ACC CAC TGT CAT CCT ATG GAC ATC CTT GGG ACT Leu Met Asp Phe Gln Thr His Cys His Pro Met Asp Ile Leu Gly Thr 610 615 620	871
TGT GCA ACT GAG CAG TCC AGA TGT CTG CGG GCA TAC CTG GGG CTG ATT Cys Ala Thr Glu Gln Ser Arg Cys Leu Arg Ala Tyr Leu Gly Leu Ile 625 630 635	919
GGG ACT GCC ATG ACC CCA AAC TTC ATC AGC AAG GTC AAC ACT ACT GTT Gly Thr Ala Met Thr Pro Asn Phe Ile Ser Lys Val Asn Thr Thr Val 640 645 650 655	967
GCC TTA AGC TGC ACC TGC CGA GGC AGC GGC AAC CTA CAG GAC GAG TGT Ala Leu Ser Cys Thr Cys Arg Gly Ser Gly Asn Leu Gln Asp Glu Cys 660 665 670	1015
GAA CAG CTG GAA AGG TCC TTC TCC CAG AAC CCC TGC CTC GTG GAG GCC Glu Gln Leu Glu Arg Ser Phe Ser Gln Asn Pro Cys Leu Val Glu Ala 675 680 685	1063
ATT GCA GCT AAG ATG CGT TTC CAC AGA CAG CTC TTC TCC CAG GAC TGG Ile Ala Ala Lys Met Arg Phe His Arg Gln Leu Phe Ser Gln Asp Trp 690 695 700	1111
GCA GAC TCT ACT TTT TCA GTG GTG CAG CAG CAG AAC AGC AAC CCT GCT Ala Asp Ser Thr Phe Ser Val Val Gln Gln Gln Asn Ser Asn Pro Ala 705 710 715	1159
CTG AGA CTG CAG CCC AGG CTA CCC ATT CTT TCT TTC TCC ATC CTT CCC Leu Arg Leu Gln Pro Arg Leu Pro Ile Leu Ser Phe Ser Ile Leu Pro 720 725 730 735	1207
TTG ATT CTG CTG CAG ACC CTC TGG TAGCTGGGCT TCCTCAGGGT CCTTTGTCCT Leu Ile Leu Leu Gln Thr Leu Trp 740	1261

CTCCACCACA CCCAGACTGA TTTGCAGCCT GTGGTGGGAG AGAACTCGCC AGCCTGTGGA 1321  
 AGAAGACGCA GCGTGCTACA CAGCAACCCG GAACCAACCA GGCATTCCGC AGCACATCCC 1381  
 GTCTGCTCCA GAAGAGGTCT TAGAAGTGAG GGCTGTGACC CTTCCGATCC TGAGCGGCTA 1441  
 GTTTTCAAAC CTCCCTTGCC CCTGCTTCCT TCTGGCTCAG GCTGCTCCTC CTTAGGACTT 1501  
 TGTGGGTCCA GTTTTGCCTT CTGTTCTGAT GGTGATTAGC GGCTCACCTC CAGCGCTTCT 1561  
 TCCTGTTTCC CAGGACCACC CAGAGGCTAA GGAATCAGTC ATTCCCTGTT GCCTTCTCCA 1621  
 GGAAGGCAGG CTAAGGGTTC TGAGGTGACT GAGAAAAATG TTTCCTTTGT GTGGAAGGCT 1681  
 GGTGCTCCAG CCTCCACGTC CCTCTGAATG GAAGATAAAA ACCTGCTGGT GTCTTGACTG 1741  
 CTCTGCCAGG CAATCCTGAA CATTGTTGGCA TGAAGAGCTA AAGTCTTTGG GTCTTGTTTA 1801  
 ACTCCTATTA CTGTCCCCAA ATTCCCCTAG TCCCTTGGGT CATGATTAAA CATTITGACT 1861  
 TAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 1889

## (2) INFORMATION FOR SEQ ID NO:17:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 397 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met Gly Leu Ser Trp Ser Pro Arg Pro Pro Leu Leu Met Ile Leu Leu  
 1 5 10 15  
 Leu Val Leu Ser Leu Trp Leu Pro Leu Gly Ala Gly Asn Ser Leu Ala  
 20 25 30  
 Thr Glu Asn Arg Phe Val Asn Ser Cys Thr Gln Ala Arg Lys Lys Cys  
 35 40 45  
 Glu Ala Asn Pro Ala Cys Lys Ala Ala Tyr Gln His Leu Gly Ser Cys  
 50 55 60  
 Thr Ser Ser Leu Ser Arg Pro Leu Pro Leu Glu Glu Ser Ala Met Ser  
 65 70 75 80  
 Ala Asp Cys Leu Glu Ala Ala Glu Gln Leu Arg Asn Ser Ser Leu Ile  
 85 90 95  
 Asp Cys Arg Cys His Arg Arg Met Lys His Gln Ala Thr Cys Leu Asp  
 100 105 110

Ile Tyr Trp Thr Val His Pro Ala Arg Ser Leu Gly Asp Tyr Glu Leu  
 115 120 125  
 Asp Val Ser Pro Tyr Glu Asp Thr Val Thr Ser Lys Pro Trp Lys Met  
 130 135 140  
 Asn Leu Ser Lys Leu Asn Met Leu Lys Pro Asp Ser Asp Leu Cys Leu  
 145 150 155 160  
 Lys Phe Ala Met Leu Cys Thr Leu His Asp Lys Cys Asp Arg Leu Arg  
 165 170 175  
 Lys Ala Tyr Gly Glu Ala Cys Ser Gly Ile Arg Cys Gln Arg His Leu  
 180 185 190  
 Cys Leu Ala Gln Leu Arg Ser Phe Phe Glu Lys Ala Ala Glu Ser His  
 195 200 205  
 Ala Gln Gly Leu Leu Leu Cys Pro Cys Ala Pro Glu Asp Ala Gly Cys  
 210 215 220  
 Gly Glu Arg Arg Arg Asn Thr Ile Ala Pro Ser Cys Ala Leu Pro Ser  
 225 230 235 240  
 Val Thr Pro Asn Cys Leu Asp Leu Arg Ser Phe Cys Arg Ala Asp Pro  
 245 250 255  
 Leu Cys Arg Ser Arg Leu Met Asp Phe Gln Thr His Cys His Pro Met  
 260 265 270  
 Asp Ile Leu Gly Thr Cys Ala Thr Glu Gln Ser Arg Cys Leu Arg Ala  
 275 280 285  
 Tyr Leu Gly Leu Ile Gly Thr Ala Met Thr Pro Asn Phe Ile Ser Lys  
 290 295 300  
 Val Asn Thr Thr Val Ala Leu Ser Cys Thr Cys Arg Gly Ser Gly Asn  
 305 310 315 320  
 Leu Gln Asp Glu Cys Glu Gln Leu Glu Arg Ser Phe Ser Gln Asn Pro  
 325 330 335  
 Cys Leu Val Glu Ala Ile Ala Ala Lys Met Arg Phe His Arg Gln Leu  
 340 345 350  
 Phe Ser Gln Asp Trp Ala Asp Ser Thr Phe Ser Val Val Gln Gln Gln  
 355 360 365  
 Asn Ser Asn Pro Ala Leu Arg Leu Gln Pro Arg Leu Pro Ile Leu Ser  
 370 375 380  
 Phe Ser Ile Leu Pro Leu Ile Leu Leu Gln Thr Leu Trp  
 385 390 395

79

## (2) INFORMATION FOR SEQ ID NO:18:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1271 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2..946

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

C GGC TAC TGT GAA ACA CCT CAA CTC AGG AAC AGC TCT CTG ATA GGC	46
Gly Tyr Cys Glu Thr Pro Gln Leu Arg Asn Ser Ser Leu Ile Gly	
400 405 410	
TGC ATG TGC CAC CGG CGC ATG AAG AAC CAG GTT GCC TGC TTG GAC ATC	94
Cys Met Cys His Arg Arg Met Lys Asn Gln Val Ala Cys Leu Asp Ile	
415 420 425	
TAT TGG ACC GTT CAC CGT GCC CGC AGC CTT GGT AAC TAT GAG CTG GAT	142
Tyr Trp Thr Val His Arg Ala Arg Ser Leu Gly Asn Tyr Glu Leu Asp	
430 435 440	
GTC TCC CCC TAT GAA GAC ACA GTG ACC AGC AAA CCC TGG AAA ATG AAT	190
Val Ser Pro Tyr Glu Asp Thr Val Thr Ser Lys Pro Trp Lys Met Asn	
445 450 455 460	
CTC AGC AAA CTG AAC ATG CTC AAA CCA GAC TCA GAC CTC TGC CTC AAG	238
Leu Ser Lys Leu Asn Met Leu Lys Pro Asp Ser Asp Leu Cys Leu Lys	
465 470 475	
TTT GCC ATG CTG TGT ACT CTC AAT GAC AAG TGT GAC CGG CTG CGC AAG	286
Phe Ala Met Leu Cys Thr Leu Asn Asp Lys Cys Asp Arg Leu Arg Lys	
480 485 490	
GCC TAC GGG GAG GCG TGC TCC GGG CCC CAC TGC CAG CGC CAC GTC TGC	334
Ala Tyr Gly Glu Ala Cys Ser Gly Pro His Cys Gln Arg His Val Cys	
495 500 505	
CTC AGG CAG CTG CTC ACT TTC TTC GAG AAG GCC GCC GAG CCC CAC GCG	382
Leu Arg Gln Leu Leu Thr Phe Phe Glu Lys Ala Ala Glu Pro His Ala	
510 515 520	
CAG GGC CTG CTA CTG TGC CCA TGT GCC CCC AAC GAC CGG GGC TGC GGG	430
Gln Gly Leu Leu Leu Cys Pro Cys Ala Pro Asn Asp Arg Gly Cys Gly	
525 530 535 540	

GAG CGC CGG CGC AAC ACC ATC GCC CCC AAC TGC GCG CTG CCG CCT GTG Glu Arg Arg Arg Asn Thr Ile Ala Pro Asn Cys Ala Leu Pro Pro Val 545 550 555	478
GCC CCC AAC TGC CTG GAG CTG CGG CGC CTC TGC TTC TCC GAC CCG CTT Ala Pro Asn Cys Leu Glu Leu Arg Arg Leu Cys Phe Ser Asp Pro Leu 560 565 570	526
TGC AGA TCA CGC CTG GTG GAT TTC CAG ACC CAC TGC CAT CCC ATG GAC Cys Arg Ser Arg Leu Val Asp Phe Gln Thr His Cys His Pro Met Asp 575 580 585	574
ATC CTA GGA ACT TGT GCA ACA GAG CAG TCC AGA TGT CTA CGA GCA TAC Ile Leu Gly Thr Cys Ala Thr Glu Gln Ser Arg Cys Leu Arg Ala Tyr 590 595 600	622
CTG GGG CTG ATT GGG ACT GCC ATG ACC CCC AAC TTT GTC AGC AAT GTC Leu Gly Leu Ile Gly Thr Ala Met Thr Pro Asn Phe Val Ser Asn Val 605 610 615 620	670
AAC ACC AGT GTT GCC TTA AGC TGC ACC TGC CGA GGC AGT GGC AAC CTG Asn Thr Ser Val Ala Leu Ser Cys Thr Cys Arg Gly Ser Gly Asn Leu 625 630 635	718
CAG GAG GAG TGT GAA ATG CTG GAA GGG TTC TTC TCC CAC AAC CCC TGC Gln Glu Glu Cys Glu Met Leu Glu Gly Phe Phe Ser His Asn Pro Cys 640 645 650	766
CTC ACG GAG GCC ATT GCA GCT AAG ATG CGT TTT CAC AGC CAA CTC TTC Leu Thr Glu Ala Ile Ala Ala Lys Met Arg Phe His Ser Gln Leu Phe 655 660 665	814
TCC CAG GAC TGG CCA CAC CCT ACC TTT GCT GTG ATG GCA CAC CAG AAT Ser Gln Asp Trp Pro His Pro Thr Phe Ala Val Met Ala His Gln Asn 670 675 680	862
GAA AAC CCT GCT GTG AGG CCA CAG CCC TGG GTG CCC TCT CTT TTC TCC Glu Asn Pro Ala Val Arg Pro Gln Pro Trp Val Pro Ser Leu Phe Ser 685 690 695 700	910
TGC ACG CTT CCC TTG ATT CTG CTC CTG AGC CTA TGG TAGCTGGACT Cys Thr Leu Pro Leu Ile Leu Leu Leu Ser Leu Trp 705 710	956
TCCCCAGGGC CCTCTTCCCC TCCACCACAC CCAGGTGGAC TTGCAGCCCA CAAGGGGTGA	1016
GGAAAGGACA GCAGCAGGAA GGAGGTGCAG TGC GCAGATG AGGGCACAGG AGAAGCTAAG	1076
GGTTATGACC TCCAGATCCT TACTGGTCCA GTCCTCATTC CCTCCACCCC ATCTCCACTT	1136
CTGATTCATG CTGCCCCCTCC TTGGTGGCCA CAATTTAGCC ATGTCATCTG GTGCCTGTGG	1196
GCCTTGCTTT ATTCTATTA TTGTCCTAAA GTCTCTCTGG GCTCTTGGAT CATGATTAAA	1256
CCTTTGACTT AAAAA	1271

## (2) INFORMATION FOR SEQ ID NO:19:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 315 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

~~(ii) MOLECULE TYPE: protein~~

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Gly	Tyr	Cys	Glu	Thr	Pro	Gln	Leu	Arg	Asn	Ser	Ser	Leu	Ile	Gly	Cys	1	5	10	15
Met	Cys	His	Arg	Arg	Met	Lys	Asn	Gln	Val	Ala	Cys	Leu	Asp	Ile	Tyr	20	25	30	
Trp	Thr	Val	His	Arg	Ala	Arg	Ser	Leu	Gly	Asn	Tyr	Glu	Leu	Asp	Val	35	40	45	
Ser	Pro	Tyr	Glu	Asp	Thr	Val	Thr	Ser	Lys	Pro	Trp	Lys	Met	Asn	Leu	50	55	60	
Ser	Lys	Leu	Asn	Met	Leu	Lys	Pro	Asp	Ser	Asp	Leu	Cys	Leu	Lys	Phe	65	70	75	80
Ala	Met	Leu	Cys	Thr	Leu	Asn	Asp	Lys	Cys	Asp	Arg	Leu	Arg	Lys	Ala	85	90	95	
Tyr	Gly	Glu	Ala	Cys	Ser	Gly	Pro	His	Cys	Gln	Arg	His	Val	Cys	Leu	100	105	110	
Arg	Gln	Leu	Leu	Thr	Phe	Phe	Glu	Lys	Ala	Ala	Glu	Pro	His	Ala	Gln	115	120	125	
Gly	Leu	Leu	Leu	Cys	Pro	Cys	Ala	Pro	Asn	Asp	Arg	Gly	Cys	Gly	Glu	130	135	140	
Arg	Arg	Arg	Asn	Thr	Ile	Ala	Pro	Asn	Cys	Ala	Leu	Pro	Pro	Val	Ala	145	150	155	160
Pro	Asn	Cys	Leu	Glu	Leu	Arg	Arg	Leu	Cys	Phe	Ser	Asp	Pro	Leu	Cys	165	170	175	
Arg	Ser	Arg	Leu	Val	Asp	Phe	Gln	Thr	His	Cys	His	Pro	Met	Asp	Ile	180	185	190	
Leu	Gly	Thr	Cys	Ala	Thr	Glu	Gln	Ser	Arg	Cys	Leu	Arg	Ala	Tyr	Leu	195	200	205	
Gly	Leu	Ile	Gly	Thr	Ala	Met	Thr	Pro	Asn	Phe	Val	Ser	Asn	Val	Asn	210	215	220	

Thr Ser Val Ala Leu Ser Cys Thr Cys Arg Gly Ser Gly Asn Leu Gln  
 225 230 235 240  
 Glu Glu Cys Glu Met Leu Glu Gly Phe Phe Ser His Asn Pro Cys Leu  
 245 250 255  
 Thr Glu Ala Ile Ala Ala Lys Met Arg Phe His Ser Gln Leu Phe Ser  
 260 265 270  
 Gln Asp Trp Pro His Pro Thr Phe Ala Val Met Ala His Gln Asn Glu  
 275 280 285  
 Asn Pro Ala Val Arg Pro Gln Pro Trp Val Pro Ser Leu Phe Ser Cys  
 290 295 300  
 Thr Leu Pro Leu Ile Leu Leu Leu Ser Leu Trp  
 305 310 315

## (2) INFORMATION FOR SEQ ID NO:20:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1699 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 175..1374

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

TGTGGACGCG CGCTTCGGAG TTGGAGGGCG GCGCCCAGGA CCCTGGTGGG AGAGTGTGTG 60  
 CGTCGCGCTG GAGGGCGGGA GGCGGGGGCG GGAGGTGCCG GTCGAGGGAG CCCCCTCTC 120  
 AGAGCTCCAG GGGAGGAGCG AGGGGAGCGC GGAGCCCGGC GCCTACAGCT CGCC ATG 177  
 Met  
 GTG CGC CCC CTG AAC CCG CGA CCG CTG CCG CCC GTA GTC CTG ATG TTG 225  
 Val Arg Pro Leu Asn Pro Arg Pro Leu Pro Pro Val Val Leu Met Leu 320 325 330  
 CTG CTG CTG CTG CCG CCG TCG CCG CTG CCT CTC GCA GCC GGA GAC CCC 273  
 Leu Leu Leu Leu Pro Pro Ser Pro Leu Pro Leu Ala Ala Gly Asp Pro 335 340 345  
 CTT CCC ACA GAA AGC CGA CTC ATG AAC AGC TGT CTC CAG GCC AGG AGG 321  
 Leu Pro Thr Glu Ser Arg Leu Met Asn Ser Cys Leu Gln Ala Arg Arg 350 355 360



AAG TGC CAG GCT GAT CCC ACC TGC AGT GCT GCC TAC CAC CAC CTG GAT	369
Lys Cys Gln Ala Asp Pro Thr Cys Ser Ala Ala Tyr His His Leu Asp	
365 370 375 380	
TCC TGC ACC TCT AGC ATA AGC ACC CCA CTG CCC TCA GAG GAG CCT TCG	417
Ser Cys Thr Ser Ser Ile Ser Thr Pro Leu Pro Ser Glu Glu Pro Ser	
385 390 395	
GTC CCT GCT GAC TGC CTG GAG GCA GCA CAG CAA CTC AGG AAC AGC TCT	465
Val Pro Ala Asp Cys Leu Glu Ala Ala Gln Gln Leu Arg Asn Ser Ser	
400 405 410	
CTG ATA GGC TGC ATG TGC CAC CGG CGC ATG AAG AAC CAG GTT GCC TGC	513
Leu Ile Gly Cys Met Cys His Arg Arg Met Lys Asn Gln Val Ala Cys	
415 420 425	
TTG GAC ATC TAT TGG ACC GTT CAC CGT GCC CGC AGC CTT GGT AAC TAT	561
Leu Asp Ile Tyr Trp Thr Val His Arg Ala Arg Ser Leu Gly Asn Tyr	
430 435 440	
GAG CTG GAT GTC TCC CCC TAT GAA GAC ACA GTG ACC AGC AAA CCC TGG	609
Glu Leu Asp Val Ser Pro Tyr Glu Asp Thr Val Thr Ser Lys Pro Trp	
445 450 455 460	
AAA ATG AAT CTC AGC AAA CTG AAC ATG CTC AAA CCA GAC TCA GAC CTC	657
Lys Met Asn Leu Ser Lys Leu Asn Met Leu Lys Pro Asp Ser Asp Leu	
465 470 475	
TGC CTC AAG TTT GCC ATG CTG TGT ACT CTC AAT GAC AAG TGT GAC CGG	705
Cys Leu Lys Phe Ala Met Leu Cys Thr Leu Asn Asp Lys Cys Asp Arg	
480 485 490	
CTG CGC AAG GCC TAC GGG GAG GCG TGC TCC GGG CCC CAC TGC CAG CGC	753
Leu Arg Lys Ala Tyr Gly Glu Ala Cys Ser Gly Pro His Cys Gln Arg	
495 500 505	
CAC GTC TGC CTC AGG CAG CTG CTC ACT TTC TTC GAG AAG GCC GCC GAG	801
His Val Cys Leu Arg Gln Leu Leu Thr Phe Phe Glu Lys Ala Ala Glu	
510 515 520	
CCC CAC GCG CAG GGC CTG CTA CTG TGC CCA TGT GCC CCC AAC GAC CGG	849
Pro His Ala Gln Gly Leu Leu Leu Cys Pro Cys Ala Pro Asn Asp Arg	
525 530 535 540	
GGC TGC GGG GAG CGC CGG CGC AAC ACC ATC GCC CCC AAC TGC GCG CTG	897
Gly Cys Gly Glu Arg Arg Arg Asn Thr Ile Ala Pro Asn Cys Ala Leu	
545 550 555	
CCG CCT GTG GCC CCC AAC TGC CTG GAG CTG CGG CGC CTC TGC TTC TCC	945
Pro Pro Val Ala Pro Asn Cys Leu Glu Leu Arg Arg Leu Cys Phe Ser	
560 565 570	

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GAC CCG CTT TGC AGA TCA CGC CTG GTG GAT TTC CAG ACC CAC TGC CAT	993
Asp Pro Leu Cys Arg Ser Arg Leu Val Asp Phe Gln Thr His Cys His	
575 580 585	
CCC ATG GAC ATC CTA GGA ACT TGT GCA ACA GAG CAG TCC AGA TGT CTA	1041
Pro Met Asp Ile Leu Gly Thr Cys Ala Thr Glu Gln Ser Arg Cys Leu	
590 595 600	
CGA GCA TAC CTG GGG CTG ATT GGG ACT GCC ATG ACC CCC AAC TTT GTC	1089
Arg Ala Tyr Leu Gly Leu Ile Gly Thr Ala Met Thr Pro Asn Phe Val	
605 610 615 620	
AGC AAT GTC AAC ACC AGT GTT GCC TTA AGC TGC ACC TGC CGA GGC AGT	1137
Ser Asn Val Asn Thr Ser Val Ala Leu Ser Cys Thr Cys Arg Gly Ser	
625 630 635	
GGC AAC CTG CAG GAG GAG TGT GAA ATG CTG GAA GGG TTC TTC TCC CAC	1185
Gly Asn Leu Gln Glu Glu Cys Glu Met Leu Glu Gly Phe Phe Ser His	
640 645 650	
AAC CCC TGC CTC ACG GAG GCC ATT GCA GCT AAG ATG CGT TTT CAC AGC	1233
Asn Pro Cys Leu Thr Glu Ala Ile Ala Ala Lys Met Arg Phe His Ser	
655 660 665	
CAA CTC TTC TCC CAG GAC TGG CCA CAC CCT ACC TTT GCT GTG ATG GCA	1281
Gln Leu Phe Ser Gln Asp Trp Pro His Pro Thr Phe Ala Val Met Ala	
670 675 680	
CAC CAG AAT GAA AAC CCT GCT GTG AGG CCA CAG CCC TGG GTG CCC TCT	1329
His Gln Asn Glu Asn Pro Ala Val Arg Pro Gln Pro Trp Val Pro Ser	
685 690 695 700	
CTT TTC TCC TGC ACG CTT CCC TTG ATT CTG CTC CTG AGC CTA TGG	1374
Leu Phe Ser Cys Thr Leu Pro Leu Ile Leu Leu Leu Ser Leu Trp	
705 710 715	
TAGCTGGACT TCCCCAGGGC CCTCTTCCCC TCCACCACAC CCAGGTGGAC TTGCAGCCCA	1434
CAAGGGGTGA GGAAAGGACA GCAGCAGGAA GGAGGTGCAG TGCGCAGATG AGGGCACAGG	1494
AGAAGCTAAG GGTTATGACC TCCAGATCCT TACTGGTCCA GTCCTCATTC CCTCCACCCC	1554
ATCTCCACTT CTGATTCATG CTGCCCCCTCC TTGGTGGCCA CAATTTAGCC ATGTCATCTG	1614
GTGCCTGTGG GCCTTGCTTT ATTCCTATTA TTGTCCTAAA GTCTCTCTGG GCTCTTGAT	1674
CATGATTAAA CCTTTGACTT AAAAA	1699

## (2) INFORMATION FOR SEQ ID NO:21:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 400 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```

Met Val Arg Pro Leu Asn Pro Arg Pro Leu Pro Pro Val Val Leu Met
 1           5           10           15

Leu Leu Leu Leu Leu Pro Pro Ser Pro Leu Pro Leu Ala Ala Gly Asp
 20           25           30

Pro Leu Pro Thr Glu Ser Arg Leu Met Asn Ser Cys Leu Gln Ala Arg
 35           40           45

Arg Lys Cys Gln Ala Asp Pro Thr Cys Ser Ala Ala Tyr His His Leu
 50           55           60

Asp Ser Cys Thr Ser Ser Ile Ser Thr Pro Leu Pro Ser Glu Glu Pro
 65           70           75           80

Ser Val Pro Ala Asp Cys Leu Glu Ala Ala Gln Gln Leu Arg Asn Ser
           85           90           95

Ser Leu Ile Gly Cys Met Cys His Arg Arg Met Lys Asn Gln Val Ala
          100          105          110

Cys Leu Asp Ile Tyr Trp Thr Val His Arg Ala Arg Ser Leu Gly Asn
          115          120          125

Tyr Glu Leu Asp Val Ser Pro Tyr Glu Asp Thr Val Thr Ser Lys Pro
          130          135          140

Trp Lys Met Asn Leu Ser Lys Leu Asn Met Leu Lys Pro Asp Ser Asp
          145          150          155          160

Leu Cys Leu Lys Phe Ala Met Leu Cys Thr Leu Asn Asp Lys Cys Asp
          165          170          175

Arg Leu Arg Lys Ala Tyr Gly Glu Ala Cys Ser Gly Pro His Cys Gln
          180          185          190

Arg His Val Cys Leu Arg Gln Leu Leu Thr Phe Phe Glu Lys Ala Ala
          195          200          205

Glu Pro His Ala Gln Gly Leu Leu Leu Cys Pro Cys Ala Pro Asn Asp
          210          215          220

Arg Gly Cys Gly Glu Arg Arg Arg Asn Thr Ile Ala Pro Asn Cys Ala
          225          230          235          240

Leu Pro Pro Val Ala Pro Asn Cys Leu Glu Leu Arg Arg Leu Cys Phe
          245          250          255

Ser Asp Pro Leu Cys Arg Ser Arg Leu Val Asp Phe Gln Thr His Cys
          260          265          270

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His Pro Met Asp Ile Leu Gly Thr Cys Ala Thr Glu Gln Ser Arg Cys  
 275 280 285

Leu Arg Ala Tyr Leu Gly Leu Ile Gly Thr Ala Met Thr Pro Asn Phe  
 290 295 300

Val Ser Asn Val Asn Thr Ser Val Ala Leu Ser Cys Thr Cys Arg Gly  
 305 310 315 320

Ser Gly Asn Leu Gln Glu Glu Cys Glu Met Leu Glu Gly Phe Phe Ser  
 325 330 335

His Asn Pro Cys Leu Thr Glu Ala Ile Ala Ala Lys Met Arg Phe His  
 340 345 350

Ser Gln Leu Phe Ser Gln Asp Trp Pro His Pro Thr Phe Ala Val Met  
 355 360 365

Ala His Gln Asn Glu Asn Pro Ala Val Arg Pro Gln Pro Trp Val Pro  
 370 375 380

Ser Leu Phe Ser Cys Thr Leu Pro Leu Ile Leu Leu Leu Ser Leu Trp  
 385 390 395 400

What is claimed is:

1 1. A purified and isolated DNA molecule having a nucleotide sequence set forth as rat  
2 retL1 cDNA, partial human retL1 cDNA, human retL1 cDNA, human retL2 cDNA, murine  
3 retL3 cDNA or human retL3 cDNA.

1 2. A DNA molecule coding for an amino acid sequence of rat RetL1, partial human  
2 RetL1, full-length human RetL1, human RetL2, murine RetL3 or human RetL3.

1 3. A purified and isolated DNA molecule for use in securing expression in a prokaryotic  
2 or eukaryotic host cell of a polypeptide product having at least a part of the primary structural  
3 conformation and the biological activity of rat RetL1, partial human RetL1, full-length human  
4 RetL1, human RetL2, murine RetL3 or human RetL3, said DNA selected from among:

5 a) the DNA molecule of rat retL1 cDNA, partial human retL1 cDNA, human retL1  
6 cDNA, human retL2 cDNA, murine retL3 cDNA, or human retL3 cDNA, or its complementary  
7 strand;

8 b) DNA molecules which hybridize under stringent conditions to the DNA molecules  
9 defined in a) or fragments thereof;

10 c) DNA molecules which, but for the degeneracy of the genetic code, would hybridize to  
11 the DNA molecules defined in a) and b).

1 4. A purified and isolated DNA molecule which is at least 80% homologous to rat retL1  
2 cDNA, partial human retL1 cDNA, human retL1 cDNA, human retL2 cDNA, murine retL3  
3 cDNA or human retL3 cDNA.

1 5. The DNA of claim 4, further characterised by encoding a protein with the biological  
2 activity of rat RetL1, partial human RetL1, full-length human RetL1, human RetL2, murine  
3 RetL3 or human RetL3.

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1 6. The recombinant DNA molecule according to claim 1, 2, 3, 4, or 5, operably linked to  
2 an expression control sequence.

1 7. A vector comprising a DNA molecule encoding rat RetL1, partial human RetL1, full-  
2 length human RetL1, human RetL2, murine RetL3, partial human RetL3 or full-length human  
3 RetL3.

1 8. A biologically functional plasmid or viral DNA vector comprising a DNA molecule  
2 according to one of claims 1, 2, 3, 4 or 5.

1 9. A prokaryotic or eukaryotic host cell stably transformed or transfected by a vector  
2 comprising a DNA molecule encoding rat RetL1, partial human RetL1, full-length human  
3 RetL1, human RetL2, murine RetL3, or human RetL3.

1 10. A process for the production of a polypeptide product having part or all of the  
2 primary structural conformation and the biological activity of rat RetL1, partial human RetL1,  
3 full-length human RetL1, human RetL2, murine RetL3, partial human RetL3 or full-length  
4 human RetL3, said process comprising:  
5 growing, under suitable culture conditions, prokaryotic or eukaryotic host cells  
6 transformed or transfected with a DNA molecule according to claim 1, 2, 3, 4 or 5 in a  
7 manner allowing expression of such polypeptide product, and recovering either rat  
8 RetL1, partial human RetL1, full-length human RetL1, human RetL2, murine RetL3 or  
9 human RetL3.

1 11. A polypeptide product of the expression in a procaryotic or eukaryotic host cell of a  
2 DNA according to claim 10.

1 12. Purified and isolated rat RetL1, partial human RetL1, full-length human RetL1,  
2 human RetL2, murine RetL3 or human RetL3, substantially free of other human proteins.

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1 13. A protein having an amino acid sequence which comprises rat RetL1, partial human  
2 RetL1, full-length human RetL1, human RetL2, murine RetL3, partial human RetL3 or full-  
3 length human RetL3.

1 14. A protein which is a variant of rat RetL1, partial human RetL1, full-length human  
2 RetL1, human RetL2, murine RetL3, partial human RetL3 or full-length human RetL3.

1 15. A soluble variant of the protein according to claim 11, 12, 13 or 14.

1 16. An IgG fusion protein comprising rat RetL1, partial human RetL1, full-length human  
2 RetL1, human RetL2, murine RetL3, partial human RetL3 or full-length human RetL3.

1 17. The protein of claim 15, fused to a toxin, imageable compound or radionuclide.

1 18. A specific monoclonal antibody to a protein of claim 11, 12, 13 or 14.

1 19. The antibody of claim 18, associated with a toxin, imageable compound or  
2 radionuclide.

1 20. A hybridoma cell line which produces a specific antibody to rat RetL1, partial  
2 human RetL1, full-length human RetL1, human RetL2, murine RetL3, partial human RetL3 or  
3 full-length human RetL3.

1 21. An antibody produced by a hybridoma of claim 20.

1 22. A method of promoting growth of new tissue or promoting survival of damaged  
2 tissue in a subject, comprising administering to the subject a therapeutically effective amount of  
3 the polypeptide of rat RetL1, partial human RetL1, full-length human RetL1, human RetL2,  
4 murine RetL3, partial human RetL3 or full-length human RetL3.

1           23. The method of claim 22, further comprising administering to the subject a  
2   therapeutically effective amount of a second polypeptide.

1           24. The method of claim 23, wherein the second polypeptide is GDNF, neurturin or a  
2   GDNF-related polypeptide.

1           25. The method of claim 22, wherein the tissue is kidney or neural tissue.

1           26. The method of claim 22, wherein the polypeptide is membrane bound.

1           27. The method of claim 22, wherein the polypeptide is soluble.

1           28. The method of claim 27, wherein the polypeptide is a fusion protein.

1           29. The method of claim 22, 23, 24, 25, 26 or 27, wherein the subject is human.

1           30. A method of inhibiting signal transduction involving a cell expressing a RetL,  
2   comprising contacting the cell with an antibody to the RetL.

1           31. A method of inhibiting signal transduction involving a cell expressing a RetL,  
2   comprising contacting the cell with a soluble Ret protein.

1           32. The method of claim 31, wherein the soluble Ret protein is a fusion protein.

1           33. A method for targeting a toxin, imageable compound or radionuclide to a cell  
2   expressing Ret, comprising contacting the cell with the RetL fusion protein of claim 17.

1           34. A method of suppressing growth of a tumor cell which expresses Ret, comprising  
2   contacting the cell with a fusion protein of RetL and a toxin or radionuclide.



1           35. The method of any of claims 30-34, wherein the cell is within a subject, and the  
2 protein is administered to the subject.

1           36. A method for targeting a toxin, imageable compound or radionuclide to a cell  
2 expressing a RetL, comprising contacting the cell with an anti-RetL antibody conjugated to a  
3 toxin, imageable compound or radionuclide.

1           37. A method of suppressing growth of a tumor cell which expresses a RetL, comprising  
2 contacting the cell with an anti-RetL antibody conjugated to a toxin or radionuclide.

1           38. The method of claim 36 or 37, wherein the cell is within a subject, and the  
2 conjugated antibody is administered to the subject.

1           39. A method for targeting a toxin, imageable compound or radionuclide to a cell  
2 expressing a RetL, comprising contacting the cell with a Ret fusion protein, wherein the Ret  
3 fusion protein comprises Ret and a toxin, imageable compound or radionuclide.

1           40. The method of claim 39, further comprising contacting the cell with GDNF,  
2 neurturin, or a GDNF-related polypeptide.

1           41. A method of suppressing growth of a tumor cell which expresses a RetL, comprising  
2 contacting the cell with a fusion protein of Ret and a toxin or radionuclide.

1           42. The method of claim 39, 40 or 41, wherein the cell is within a subject, and the  
2 protein is administered to the subject.

1           43. A method of treating a subject with a disorder of RetL metabolism, comprising  
2 administering to the subject a vector of claim 7 or 8.

1           44. A method of promoting growth of new tissue in a subject, comprising administering  
2 to the subject a vector of claim 7 or 8.

- 1 45. The method of claim 44, wherein the tissue is renal tissue or neural tissue.
- 1 46. A method of promoting survival of damaged tissue in a subject, comprising  
2 administering a therapeutically effective amount of a vector of claim 7 or 8 to the subject.
- 1 47. The method of claim 46, wherein the tissue is renal tissue or neural tissue.

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201 AGACCGGGCGGCGGCTTTGGATTTTGGGGGGGCGGGGACCAGCTGCGCGG 250  
251 CGGCACCATGTTCTAGCCACTCTGTACTTCGCGCTGCCACTCCTGGATT 300  
M F L A T L Y F A L P L L D L  
301 TGCTGATGTCCGCCGAGGTGAGTGGTGGAGACCGTCTGGACTGTGTGAAA 350  
L M S A E V S G G D R L D C V K  
351 GCCAGCGATCAGTGCCTGAAGGAACAGAGCTGCAGCACCAAGTACCGCAC 400  
A S D Q C L K E Q S C S T K Y R T  
401 ACTAAGGCASTGCGTGGCGGGCAAGGAAACCAACTTCAGCCTGACATCCG 450  
L R Q C V A G K E T N F S L T S G  
451 GCCTTGAGGCCAAGGATGAGTGCCGTAGCGCCATGGAGGCCTTGAAGCAG 500  
L E A K D E C R S A M E A L K Q  
501 AAGTCTCTGTACAACCTGCCGCTGCAAGCGGGGCATGAAGAAAGAGAAGAA 550  
K S L Y N C R C K R G M K K E K N  
551 TTGTCTGCGTATCTACTGGAGCATGTACCAGAGCCTGCAGGGAAATGACC 600  
C L R I Y W S M Y Q S L Q G N D L  
601 TCCTGGAAGATTCCCCGTATGAGCCGTTAACAGCAGGTTGTCAGATATA 650  
L E D S P Y E P V N S R L S D I  
651 TTCCGGGCAGTCCCGTTTCATATCAGATGTTTTCCAGCAAGTGGAAACACAT 700  
F R A V P F I S D V F Q Q V E H I  
701 TTCCAAAGGGAACAACCTGCCTGGACGCAGCCAAGGCCTGCAACCTGGACG 750  
S K G N N C L D A A K A C N L D D  
751 ACACCTGTAAGAAGTACAGGTGCGCCTACATCACCCCTGCACCACCAGC 800  
T C K K Y R S A Y I T P C T T S  
801 ATGTCCAACGAGGTCTGCAACCGCCGTAAGTGCCACAAGGCCCTCAGGCA 850  
M S N E V C N R R K C H K A L R Q  
851 GTTCTTCGACAAGGTTCCGGCCAAGCACAGCTACGGGATGCTCTTCTGCT 900  
F F D K V P A K H S Y G M L F C S  
901 CCTGCCGGGACATCGCCTGCACCGAGCGGCGGCGACAGACTATCGTCCCC 950  
C R D I A C T E R R R Q T I V P  
951 GTGTGCTCCTATGAAGAACGAGAGAGGCCCAACTGCCTGAGTCTGCAAGA 1000  
V C S Y E E R E R P N C L S L Q D  
1001 CTCCTGCAAGACCAATTACATCTGCAGATCTCGCCTTGCAATTTTTTTA 1050  
S C K T N Y I C R S R L A D F F T  
1051 CCAACTGCCAGCCAGAGTCAAGGTCTGTCAGCAACTGTCTTAAGGAGAAC 1100  
N C Q P E S R S V S N C L K E N  
1101 TACGCAGACTGCCTCCTGGCCTACTCGGGACTGATTGGCAGTCATGAC 1150  
Y A D C L L A Y S G L I G T V M T

FIG. 1a

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1151 TCCCAACTACGTAGACTCCAGCAGCCTCAGCGTGGCACCATGGTGTGACT 1200  
P N Y V D S S S L S V A P W C D C

1201 GCAGCAACAGCGGCAATGACCTGGAAGACTGCTTGAAATTTCTGAATTTT 1250  
S N S G N D L E D C L K F L N F

1251 TTTAAGGACAATACTTGTCTCAAAAATGCAATTCAAGCCTTTGGCAATGG 1300  
F K D N T C L K N A I Q A F G N G

1301 CTCAGATGTGACCATGTGGCAGCCAGCCCCTCCAGTCCAGACCACCACTG 1350  
S D V T M W Q P A P P V Q T T T A

1351 TCCACCACTACCACTGCCTTCCGGGTCAAGAACAAGCCTCTGGGGCCAGCA 1400  
T T T T A F R V K N K P L G P A

1401 GGGTCTGAGAATGAGATCCCCACACACGTTTTACCACCCTGTGCGAATTT 1450  
G S E N E I P T H V L P P C A N L

1451 GCAGGCTCAGAAGCTGAAATCCAATGTGTCTGGGTAGCACACACCTCTGTC 1500  
Q A Q K L K S N V S G S T H L C L

1501 TTTCTGATAGTGATTTTCGGAAGGATGGTCTCGCTGGTGCCTCCAGCCAC 1550  
S D S D F G K D G L A G A S S H

1551 ATAACCACAAAATCAATGGCTGCTCCTCCCAGCTGCAGTCTGAGCTCACT 1600  
I T T K S M A A P P S C S L S S L

1601 GCCGGTGCTGATGCTCACCGCCCTTGCTGCCCTGTTATCTGTATCGTTGG 1650  
P V L M L T A L A A L L S V S L A

1651 CAGAAACGTCGTAGCTGCATCCGGGAAAACAGTATGAAAAGACAAAAGAG 1700  
E T S

FIG. 1b

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1	CTGCTGGAGGATTCCCCATATGAACCAAGTTAACAGCAGATTGTCAGATAT	50
	L L E D S P Y E P V N S R L S D I	
51	ATTCCGGGTGGTCCCATTCATATCAGTGGAGCACATTCCCAAAGGGAACA	100
	F R V V P F I S V E H I P K G N N	
101	ACTGCCTGGATGCAGCGAAGGCCTGCAACCTCGACGACATTTGCAAGAAG	150
	C L D A A K A C N L D D I C K K	
151	TACAGGTCGGCGTACATCACCCCGTGCACCACCAGCGTGTCCAACGATGT	200
	<del>Y R S A Y I T P C T T S V S N D V</del>	
201	CTGCAACCGCCGCAAGTGCCACAAGGCCCTCCGGCAGTTCTTTGACAAGG	250
	C N R R K C H K A L R Q F F D K V	
251	TCCCGGCCAAGCACAGCTACGGAATGCTCTTCTGCTCCTGCCGGGACATC	300
	P A K H S Y G M L F C S C R D I	
301	GCCTGCACAGAGCGGAGGCGACAGACCATCGTGCCTGTGTGCTCCTATGA	350
	A C T E R R R Q T I V P V C S Y E	
351	AGAGAGGGAGAAGCCCAACTGTTTGAATTTGCAGGACTCCTGCAAGACGA	400
	E R E K P N C L N L Q D S C K T N	
401	ATTACATCTGCAGATCTCGCCTTGCGGATTTTTTTTACCAACTGCCAGCCA	450
	Y I C R S R L A D F F T N C Q P	
451	GAGTCAAGGTCTGTCAGCAGCTGTCTAAAGGAAAACACTACGCTGACTGCCT	500
	E S R S V S S C L K E N Y A D C L	
501	CCTCGCCTACTCGGGGCTTATTGGCACAGTCATGACCCCAACTACATAG	550
	L A Y S G L I G T V M T P N Y I D	
551	ACTCCAGTAGCCTCAGTGTGGCCCCATGGTGTGACTGCAGCAACAGTGGG	600
	S S S L S V A P W C D C S N S G	
601	AACGACCTAGAAGAGTGCTTGAAATTTTTGAATTTCTTCAAGGACAATAC	650
	N D L E E C L K F L N F F K D N T	
651	ATGTCTTAAAAATGCAATTCAAGCCTTTGGCAATGGCTCCGATGTGACCG	700
	C L K N A I Q A F G N G S D V T V	
701	TGTGGCAGCCAGCCTTCCCAGTACAGACCACCACTGCCACTACCACCACT	750
	W Q P A F P V Q T T T A T T T T	
751	GCCCTCCGGGTTAAGAACAAGCCCCTGGGGCCAGCAGGGTCTGAGAATGA	800
	A L R V K N K P L G P A G S E N E	
801	AATTCCTCATGTGTTTGGCCACCGTGTGCAAATTTACAGGCACAGAAGC	850
	I P T H V L P P C A N L Q A Q K L	
851	TGAAATCCAATGTGTGCGGGCAATACACACCTCTGTATTTCCAATGGTAAT	900
	K S N V S G N T H L C I S N G N	

FIG. 2A-1

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901 TATGAAAAAGAAGGTCTCGGTGCTTCCAGCCACATAACCACAAAATCAAT 950  
Y E K E G L G A S S H I T T K S M

951 GGCTGCTCCTCCAAGCTGTGGTCTGAGCCCACTGCTGGTCCTGGTGGTAA 1000  
A A P P S C G L S P L L V L V V T

1001 CCGCTCTGTCCACCCTATTATCTTTAACAGAAACATCATAGCTGCATTAA 1050  
A L S T L L S L T E T S

1051 AAAAATACAATATGGACATGTAAAAAGACAAAACCAAGTTATCTGTTTC 1100

1101 CTGTTCTCTTGTATAGCTGAAATTCCAGTTTAGGAGCTCAGTTGAGAAAC 1150

1151 AGTTCCATTCAACTGGAACATTTTTTTTTTTTTCCTTTTAAGAAAGCTTCT 1200

1201 TGTGATCCTTCGGGGCTTCTGTG 1223

FIG. 2A-2

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1	GGGCGGCCAGAGCAGCACAGCTGTCCGGGGATCGCTGCATGCTGAGCTCC	50
51	CTCGGCAAGACCCAGCGGCGGCTCGGGATTTTTTTGGGGGGCGGGGACC	100
101	AGCCCCGCGCCGGCACCATGTTCTGGCGACCCTGTACTTCGCGCTGCCG M F L A T L Y F A L P	150
151	CTCTTGGACTTGCTCCTGTGCGCCGAAGTGAGCGGCGGAGACCGCCTGGA L L D L L L S A E V S G G D R L D	200
201	TTGCGTGAAAGCCAGTGATCAGTGCCTGAAGGAGCAGAGCTGCAGCACCA C V K A S D Q C L K E Q S C S T K	250
251	AGTACCGCACGCTAAGGCAGTGCGTGGCGGGCAAGGAGACCAACTTCAGC Y R T L R Q C V A G K E T N F S	300
301	CTGGCATCCGGCCTGGAGGCCAAGSATGAGTGCCGCGAGCGCCATGGAGGC L A S G L E A K D E C R S A M E A	350
351	CCTGAAGCAGAAGTCGCTCTACAACTGCCGCTGCAAGCGGGGTATGAAGA L K Q K S L Y N C R C K R G M K K	400
401	AGGAGAAGAACTGCCTGCGCATTCTACTGGAGCATGTACCAGAGCCTGCAG E K N C L R I Y W S M Y Q S L Q	450
451	GGAAATGATCTGCTGGAGGATTCCCCATATGAACCAGTTAACAGCAGATT G N D L L E D S P Y E P V N S R L	500
501	GTCAGATATATTCCGGGTGGTCCCATTCATATCAGTGGAGCACATTCCCA S D I F R V V P F I S V E H I P K	550
551	AAGGGAACAACCTGCCTGGATGCAGCGAAGGCCTGCAACCTCGACGACATT G N N C L D A A K A C N L D D I	600
601	TGCAAGAAGTACAGGTCGGCGTACATCACCCCGTGCACCACCAGCGTGTG C K K Y R S A Y I T P C T T S V S	650
651	CAACGATGTCTGCAACCGCCGCAAGTGCCACAAGGCCCTCCGGCAGTTCT N D V C N R R K C H K A L R Q F F	700
701	TTGACAAGGTCCCGGCCAAGCACAGCTACGGAATGCTCTTCTGCTCCTGC D K V P A K H S Y G M L F C S C	750
751	CGGGACATCGCCTGCACAGAGCGGAGGCGACAGACCATCGTGCCTGTGTG R D I A C T E R R R Q T I V P V C	800
801	CTCCTATGAAGAGAGGGAGAAGCCCAACTGTTTGAATTTGCAGGACTCCT S Y E E R E K P N C L N L Q D S C	850
851	GCAAGACGAATTACATCTGCAGATCTCGCCTTGCGGATTTTTTTTACCAAC K T N Y I C R S R L A D F F T N	900
901	TGCCAGCCAGAGTCAAGGTCTGTCAGCAGCTGTCTAAAGGAAAACCTACGC C Q P E S R S V S S C L K E N Y A	950

FIG. 2B-1

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951 TGA CTGCCTCCTCGCCTACTCGGGGCTTATTGGGCACAGTCATGACCCCCA 1000  
D C L L A Y S G L I G T V M T P N

1001 ACTACATAGACTCCAGTAGCCTCAGTGTGGCCCCATGGTGTGACTGCAGC 1050  
Y I D S S S L S V A P W C D C S

1051 AACAGTGGGAACGACCTAGAAAGAGTGCTTGAAATTTTGAATTTCTTCAA 1100  
N S G N D L E E C L K F L N F F K

1101 GGACAATACATGTCTTAAAAATGCAATTCAAGCCTTTGGCAATGGCTCCG 1150  
D N T C L K N A I Q A F G N G S D

1151 ATGTGACCGTGTGGCAGCCAGCCTTCCCAGTACAGACCACCACTGCCACT 1200  
V T V W Q P A F P V Q T T T A T

1201 ACCACCACTGCCCTCCGGGTTAAGAACAAGCCCCTGGGGCCAGCAGGGTC 1250  
T T T A L R V K N K P L G P A G S

1251 TGAGAATGAAATTCCCACTCATGTTTTGCCACCGTGTGCAAATTTACAGG 1300  
E N E I P T H V L P P C A N L Q A

1301 CACAGAAGCTGAAATCCAATGTGTGGGCAATACACACCTCTGTATTTCC 1350  
Q K L K S N V S G N T H L C I S

1351 AATGGTAATTATGAAAAAGAAGGTCTCGGTGCTTCCAGCCACATAACCCAC 1400  
N G N Y E K E G L G A S S H I T T

1401 AAAATCAATGGCTGCTCCTCCAAGCTGTGGTCTGAGCCCCTGCTGGTCC 1450  
K S M A A P P S C G L S P L L V L

1451 TGGTGGTAACCGCTCTGTCCACCCTATTATCTTTAACAGAAACATCATAG 1500  
V V T A L S T L L S L T E T S

1501 CTGCATTAAAAAATAACAATATGGACATGTAAAAAGACAAAAACCAAGTT 1550

1551 ATCTGTTTCCTGTTCTCTTGTATAGCTGAAATTCAGTTTAGGAGCTCAG 1600

1601 TTGAGAAACAGTTCCATTCAACTGGAACATTTTTTTTTTTTCTTTTAAG 1650

1651 AAAGCTTCTTGTGATCCTTCGGGGCTTCTGTG 1682

FIG. 2B-2

SUBSTITUTE SHEET (RULE 26)



1 GGGCGGCCAGAGCAGCACAGCTGTCCGCGGATCGCTGCATGCTGAGCTCC 50  
| | | | |  
143 GAGTGGCCAGAGGAGCGCAGTCGCCCGGGGATCGCTGCACGCTGAGCTCT 192  
| | | | |  
51 CTCGGCAAGAACCAGCGGCGGCTCGGGATTTTTTTTGGGGGGGCGGGGACC 100  
| | | | |  
193 CTCCCCGAGACCGGGCGGCGGCTTTGGA... TTTTGGGGGGGCGGGGACC 239  
| | | | |  
101 AGCCCCGCGCCGGCACCATGTTTCCTGGCGACCCTGTACTTCGCGCTGCCG 150  
| | | | |  
240 AGCTGCGCGGCGGCACCATGTTTCCTAGCCACTCTGTACTTCGCGCTGCCA 289  
| | | | |  
151 CTCTTGGACTIONGCTCCTGTCCGCCGAAGTGAGCGGCGGAGACCGCCTGGA 200  
| | | | |  
290 CTCCTGGATTGCTGATGTCCGCCGAGGTGAGTGGTGGAGACCGTCTGGA 339  
| | | | |  
201 TTGCGTGAAAGCCAGTGATCAGTGCCCTGAAGGAGCAGAGCTGCAGCACCA 250  
| | | | |  
340 CTGTGTGAAAGCCAGCGATCAGTGCCCTGAAGGAACAGAGCTGCAGCACCA 389  
| | | | |  
251 AGTACCGCACGCTAAGGCAGTGCGTGCGGGCAAGGAGACCAACTTCAGC 300  
| | | | |  
390 AGTACCGCACACTAAGGCAGTGCGTGCGGGCAAGGAAACCAACTTCAGC 439  
| | | | |  
301 CTGGCATCCGSCCTGGAGGCCAAGGATGAGTGCCCGAGCGCCATGGAGGC 350  
| | | | |  
440 CTGACATCCGSCCTTGAGGCCAAGGATGAGTGCCGTAGCGCCATGGAGGC 489  
| | | | |  
351 CCTGAAGCAGAAGTCGCTCTACAAGTCCCCTGCAAGCGGGGTATGAAGA 400  
| | | | |  
490 CTTGAAGCAGAAGTCTCTGTACAAGTCCCCTGCAAGCGGGGCATGAAGA 539  
| | | | |  
401 AGGAGAAGAACTGCCTGCGCATTTACTGGAGCATGTACCAGAGCCTGCAG 450  
| | | | |  
540 AAGAGAAGAAATTGTCTGCGTATCTACTGGAGCATGTACCAGAGCCTGCAG 589  
| | | | |  
451 GGAAATGATCTGCTGGAGGATTCCCCATATGAACCAGTTAACAGCAGATT 500  
| | | | |  
590 GGAAATGACCTCCTGGAAGATTCCCCGTATGAGCCGGTTAACAGCAGGTT 639  
| | | | |  
501 GTCAGATATATTCCGGGTGGTCCCATTTCATATC.....AG 535  
| | | | |  
640 GTCAGATATATTCCGGGCAGTCCCGTTTCATATCAGATGTTTTCCAGCAAG 689  
| | | | |  
536 TGGAGCACATTCCCAAAGGGAACAAGTGCCTGGATGCAGCGAAGGCCTGC 585  
| | | | |  
690 TGGAACACATTTCCCAAAGGGAACAAGTGCCTGGACGCAGCCAAGGCCTGC 739  
| | | | |  
586 AACCTCGACGACATTTGCAAGAAGTACAGGTGGCGGTACATCACCCCGTG 635  
| | | | |  
740 AACCTGGACGACACTGTAAGAAGTACAGGTGGCGGTACATCACCCCTG 789  
| | | | |  
636 CACCACCAGCGTGTCCAACGATGTCTGCAACCGCCGCAAGTGCCACAAGG 685  
| | | | |  
790 CACCACCAGCATGTCCAACGAGGTCTGCAACCGCCGTAAGTGCCACAAGG 839  
| | | | |

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686 CCCTCCGGCAGTTCTTTGACAAGGTCCCGGCCAAGCACAGCTACGGAATG 735  
|||  
840 CCCTCAGGCAGTTCTTCGACAAGGTTCGGCCAAGCACAGCTACGGGATG 889  
|||  
736 CTCTTCTGCTCCTGCCGGGACATCGCCTGCACAGAGCGGAGGCGACAGAC 785  
|||  
890 CTCTTCTGCTCCTGCCGGGACATCGCCTGCACCGAGCGGCGGCGACAGAC 939  
|||  
786 CATCGTGCCTGTGTGCTCCTATGAAGAGAGGGAGAAGCCCAACTGTTTGA 835  
|||  
940 TATCGTCCCCGTGTGTGCTCCTATGAAGAACGAGAGAGGCCCAACTGCCTGA 989  
|||  
836 ATTTGCAGGACTCCTGCAAGACGAATTACATCTGCAGATCTCGCCTTGCG 885  
|||  
990 GTCTGCAAGACTCCTGCAAGACCAATTACATCTGCAGATCTCGCCTTGCA 1039  
|||  
886 GATTTTTTTTACCAACTGCCAGCCAGAGTCAAGGTCTGTCAGCAGCTGTCT 935  
|||  
1040 GATTTTTTTTACCAACTGCCAGCCAGAGTCAAGGTCTGTCAGCAACTGTCT 1089  
|||  
936 AAAGGAAAACACTACGCTGACTGCCTCCTCGCCTACTCGGGGCTTATTGGCA 985  
|||  
1090 TAAGGAGAACTACGCAGACTGCCTCCTGGCCTACTCGGGACTGATTGGCA 1139  
|||  
986 CAGTCATGACCCCCAACTACATAGACTCCAGTAGCCTCAGTGTGGCCCCA 1035  
|||  
1140 CAGTCATGACTCCCAACTACGTAGACTCCAGCAGCCTCAGCGTGGCACCA 1189  
|||  
1036 TGGTGTGACTGCAGCAACAGTGGGAACGACCTAGAAGAGTGCTTGAAATT 1085  
|||  
1190 TGGTGTGACTGCAGCAACAGCGGCAATGACCTGGAAGACTGCTTGAAATT 1239  
|||  
1086 TTTGAATTTCTTCAAGGACAATACATGTCTTAAAAATGCAATTCAAGCCT 1135  
|||  
1240 TCTGAATTTTTTTAAGGACAATACTTGTCTCAAAAATGCAATTCAAGCCT 1289  
|||  
1136 TTGGCAATGGCTCCGATGTGACCGTGTGGCAGCCAGCCTTCCCAGTACAG 1185  
|||  
1290 TTGGCAATGGCTCAGATGTGACCATGTGGCAGCCAGCCCCTCCAGTCCAG 1339  
|||  
1186 ACCACCACTGCCACTACCACCACTGCCCTCCGGGTAAAGAACAAAGCCCCT 1235  
|||  
1340 ACCACCACTGCCACTACCACCTGCCCTCCGGGTCAAGAACAAAGCCTCT 1389  
|||  
1236 GGGGCCAGCAGGGTCTGAGAATGAAATTCCCACTCATGTTTTGCCACCGT 1285  
|||  
1390 GGGGCCAGCAGGGTCTGAGAATGAGATCCCCACACACGTTTTACCACCCT 1439  
|||  
1286 GTGCAAATTTACAGGCACAGAAGCTGAAATCCAATGTGTGCGGGCAATACA 1335  
|||  
1440 GTGCGAATTTGCAGGCTCAGAAGCTGAAATCCAATGTGTGCGGGTAGCACA 1489  
|||  
1336 CACCTCTGTATTTCCAATGGTAATTATGAAAAAGAAGGTCTC...GGTGC 1382  
|||  
1490 CACCTCTGTCTTTCTGATAGTGATTTTCGAAAGGATGGTCTCGCTGGTGC 1539  
|||

FIG. 3A-2

SUBSTITUTE SHEET (RULE 26)

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1383 TTCCAGCCACATAACCACAAAATCAATGGCTGCTCCTCCAAGCTGTGGTC 1432  
|||||  
1540 CTCCAGCCACATAACCACAAAATCAATGGCTGCTCCTCCCAGCTGCAGTC 1589  
|||||  
1433 TGAGCCCCTGCTGGTCCTGGTGGTAACCGCTCTGTCCACCCTATTATCT 1482  
|||||  
1590 TGAGCTCACTGCCGGTGCTGATGCTCACCGCCCTTGCTGCCCTGTTATCT 1639  
|||||  
1483 TTAACAGAAACATCATAGCTGCATTAAAAAATACAATATGGAC 1526  
|||||  
1640 GTATCGTTGGCAGAAACGTCGTAGCTGCATCCGGGAAA.ACAGTATG... 1685  
|||||  
1527 ATGTAAAAAGACAAA...AACCAAGTTATCTGTTTCCTGTTCTCTTGTA 1572  
|||||  
1686 ....AAAAGACAAAAGAGAACCAAGTATTCTG.TCCCTGTCCTCTTGTA 1729  
|||||  
1573 TAGCTGAAATTCCAG.TTTAGGAGCTCAGTTGAGAAACAGTTCCATTCAA 1621  
|||||  
1730 TATCTGAAAATCCAGTTTTTAAAGCTCCGTTGAGAAGCAGTTTCACCCAA 1779  
|||||  
1622 CTGGAACATTTTTTTTTTTTTCCTTTTAAGAAAGCTTCTTGATCCTTCG 1671  
|||||  
1780 CTGGAAC...TCTTTCCTTGTTTTTAAGAAAG...CTTGTGGCCCTCAG 1822  
|||||  
1672 GGGCTTCTGT 1681  
|||||  
1823 GGGCTTCTGT 1832

FIG. 3A-3

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```

1  MFLATLYFALPLDLLLSAEVSGGDRLCDVKASDQCLKEQSCSTKYRTL 50
   |||||:|||||
1  MFLATLYFALPLDLLMSAEVSGGDRLCDVKASDQCLKEQSCSTKYRTL 50
   |||||:|||||
51 QCVAGKETNFSLASGLEAKDECRSAMEALKQKSLYNCRCKRGMKKEKNCL 100
   |||||:|||||
51 QCVAGKETNFSLTSGLEAKDECRSAMEALKQKSLYNCRCKRGMKKEKNCL 100
   |||||:|||||
101 RIYWSMYQSLQGNLLEDSPYEPVNSRLSDIFRVVPFIS.....VEHIPK 145
   |||||:|||||
101 RIYWSMYQSLQGNLLEDSPYEPVNSRLSDIFRAVPFISDVFQQVEHISK 150
   |||||:|||||
146 GNNCLDAAKACNLDDICKKYRSAYITPCTTSVSNDCNRRKCHKALRQFF 195
   |||||:|||||
151 GNNCLDAAKACNLDDTCKKYRSAYITPCTTSMNEVCNRRKCHKALRQFF 200
   |||||:|||||
196 DKVPAKHSYGMLFCSCRDIACERRRQTI VPVCSYEEREKPNCLNLQDSC 245
   |||||:|||||
201 DKVPAKHSYGMLFCSCRDIACERRRQTI VPVCSYEERERPNCLSLQDSC 250
   |||||:|||||
246 KTN YICRSLADFFTNCPESRSVSSCLKENYADCLLAYSGLIGTVMTPN 295
   |||||:|||||
251 KTN YICRSLADFFTNCPESRSVSNCLKENYADCLLAYSGLIGTVMTPN 300
   |||||:|||||
296 YIDSSSLSVAPWCDCSNSGNDLEECLKFLNFFKDNTCLKNAIQAFNGSD 345
   |||||:|||||
301 YVDSSSLSVAPWCDCSNSGNDLEDCLKFLNFFKDNTCLKNAIQAFNGSD 350
   |||||:|||||
346 VTVWQPAFPVQTTTATTTTALRVKNKPLGPAGSENEIPTHVLPPCANLQA 395
   |||||:|||||
351 VTMWQPAFPVQTTTATTTTAFRVKNKPLGPAGSENEIPTHVLPPCANLQA 400
   |||||:|||||
396 QKLKSNVSGNTHLCISNGNYEKEGL.GASSHITTKSMAAPPSCGLSPLL 444
   |||||:|||||
401 QKLKSNVSGSTHLCISDSDFGKDGLAGASSHITTKSMAAPPSCSLSSLPV 450
   |||||:|||||
445 LVVTALSTLL..SLTETS 460
   |||||:|||||
451 LMLTALAALLSVSLAETS 468

```

FIG. 3B

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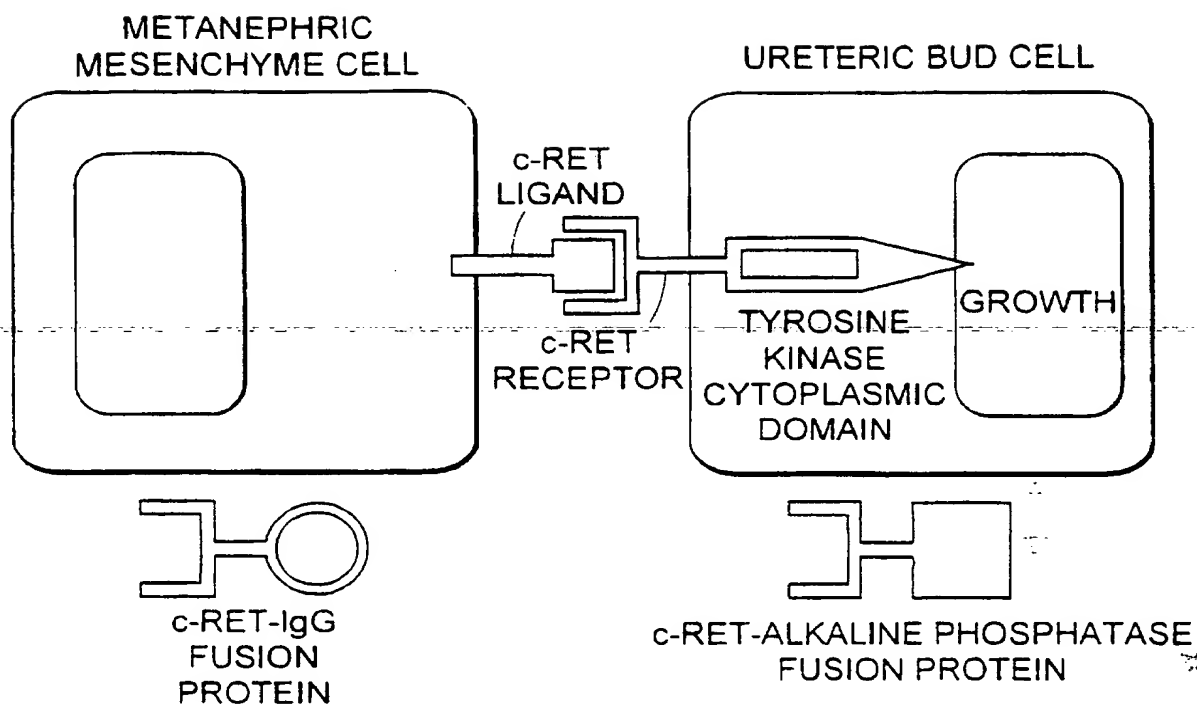


FIG. 4A

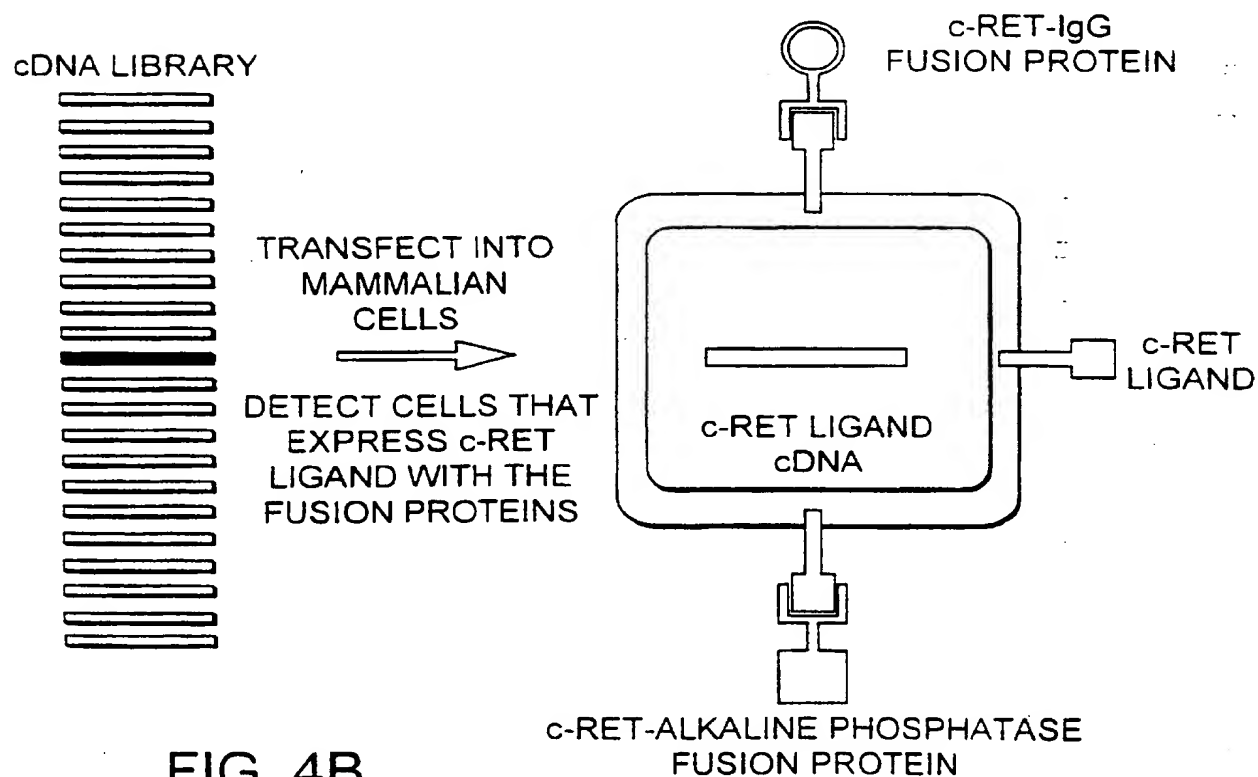


FIG. 4B

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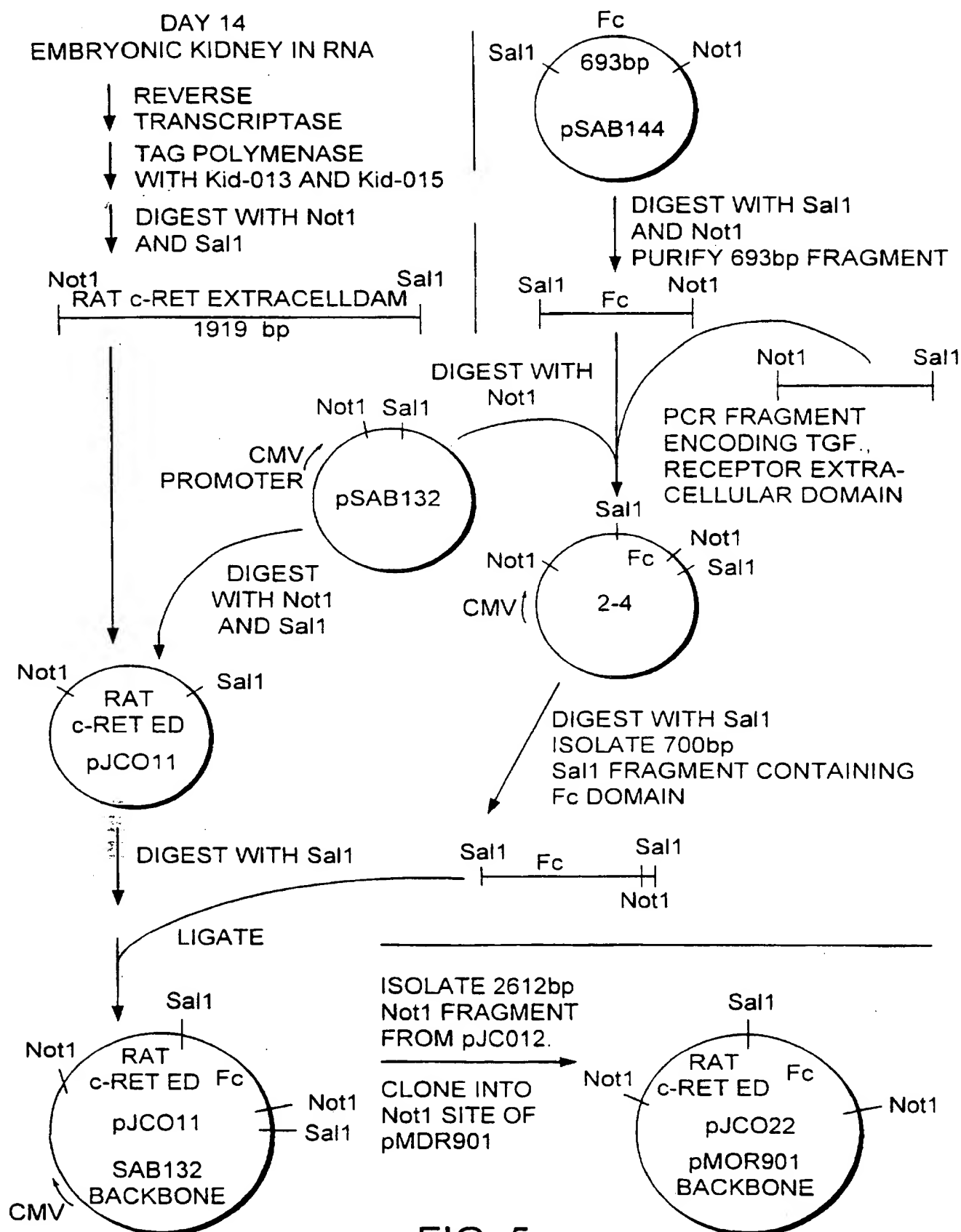


FIG. 5

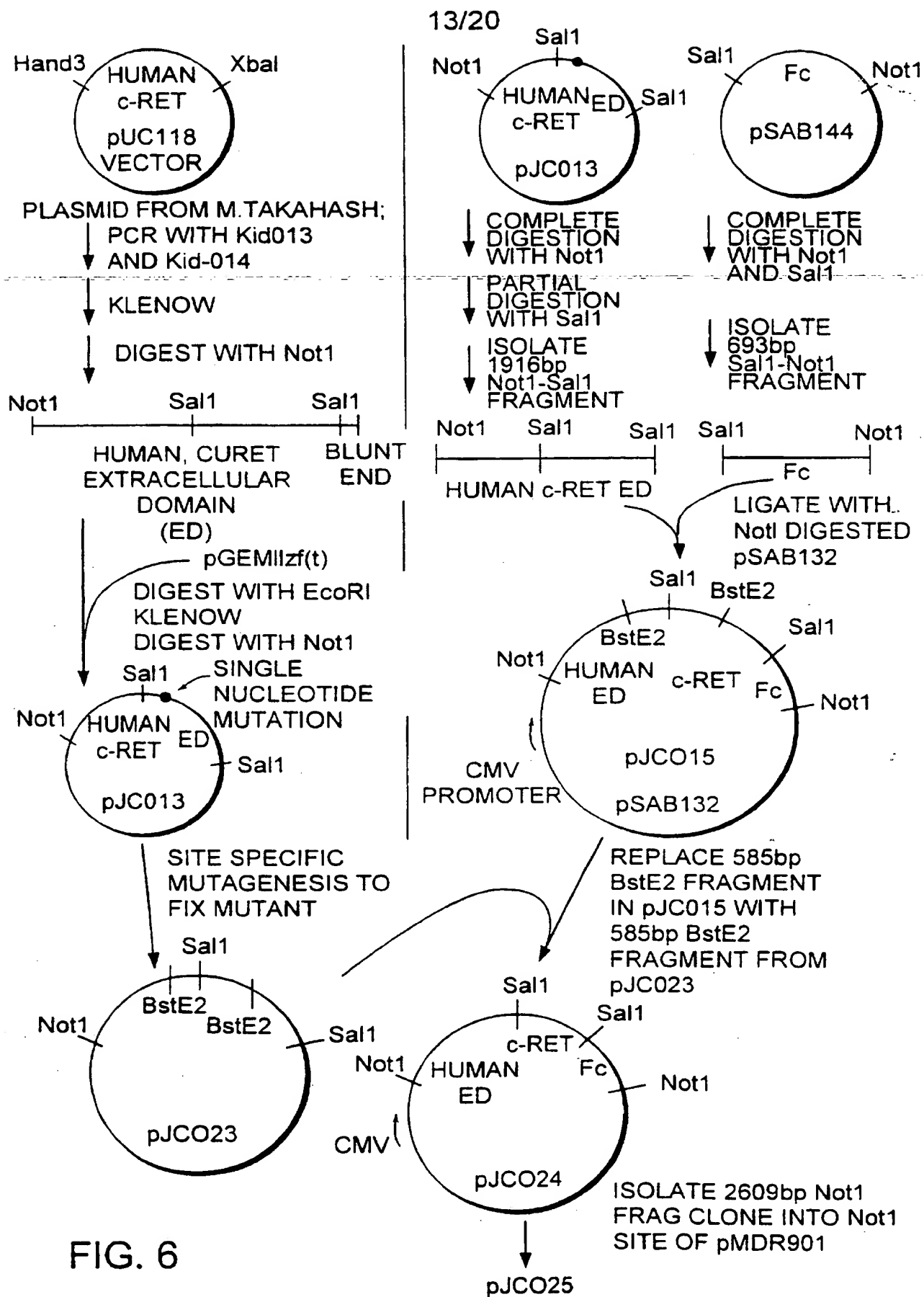


FIG. 6

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1 AAAAAACGGTGGGATTTATTTAACATGATCTTGGCAAACGTCTTCTGCCT 50  
M I L A N V F C L

51 CTTCTTCTTTCTAGACGAGACCTCCGCTCTTTGGCCAGCCCTTCCTCCC 100  
F F F L D E T L R S L A S P S S L

101 TGCAGGGCCCCGAGCTCCACGGCTGGCGCCCCCAGTGGACTGTGTCCGG 150  
Q G P E L H G W R P P V D C V R

151 GCCAATGAGCTGTGTGCCGCGAATCCAACCTGCAGCTCTCGCTACCGCAC 200  
A N E L C A A E S N C S S R Y R T

201 TCTGCGGCAGTGCCTGGCAGGCCGCGACCGCAACACCATGCTGGCCAACA 250  
L R Q C L A G R D R N T M L A N K

251 AGGAGTGCCAGGCGGCCTTGGAGGTCTTGCAGGAGAGCCCGCTGTACGAC 300  
E C Q A A L E V L Q E S P L Y D

301 TGCCGCTGCAAGCGGGGCATGAAGAAGGAGCTGCAGTGTCTGCAGATCTA 350  
C R C K R G M K K E L Q C L Q I Y

351 CTGGAGCATCCACCTGGGGCTGACCGAGGGTGAGGAGTTCTACGAAGCCT 400  
W S I H L G L T E G E E F Y E A S

401 CCCCCTATGAGCCGGTGACCTCCCGCCTCTCGGACATCTTCAGGCTTGCT 450  
P Y E P V T S R L S D I F R L A

451 TCAATCTTCTCAGGGACAGGGGCAGACCCGGTGGTCAGCGCCAAGAGCAA 500  
S I F S G T G A D P V V S A K S N

501 CCATTGCCTGGATGCTGCCAAGGCCTGCAACCTGAATGACAACCTGCAAGA 550  
H C L D A A K A C N L N D N C K K

551 AGCTGCGCTCCTCCTACATCTCCATCTGCAACCGCGAGATCTCGCCCACC 600  
L R S S Y I S I C N R E I S P T

601 GAGCGCTGCAACCGCCGCAAGTGCCACAAGGCCCTGCGCCAGTTCTTCGA 650  
E R C N R R K C H K A L R Q F F D

651 CCGGGTGCCCAGCGAGTACACCTACCGCATGCTCTTCTGCTCCTGCCAAG 700  
R V P S E Y T Y R M L F C S C Q D

701 ACCAGGCGTGCGCTGAGCGCCGCGGCAAACCATCCTGCCAGCTGCTCC 750  
Q A C A E R R R Q T I L P S C S

751 TATGAGGACAAGGAGAAGCCCAACTGCCTGGACCTGCGTGGCGTGTGCCG 800  
Y E D K E K P N C L D L R G V C R

801 GACTGACCACCTGTGTGCGGTCCCGGCTGGCCGACTTCCATGCCAATTGTC 850  
T D H L C R S R L A D F H A N C R

FIG. 7a

SUBSTITUTE SHEET (RULE 26)



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851 GAGCCTCCTACCAGACGGTCACCAGCTGCCCTGCGGACAATTACCAGGCG 900  
A S Y Q T V T S C P A D N Y Q A

901 TGTCTGGGCTCTTATGCTGGCATGATTGGGTTTGACATGACACCTAACTA 950  
C L G S Y A G M I G F D M T P N Y

951 TGTGGACTCCAGCCCCACTGGCATCGTGGTGTCCCCCTGGTGCAGCTGTC 1000  
V D S S P T G I V V S P W C S C R

1001 GTGGCAGCGGGAACATGGAGGAGGAGTGTGAGAAGTTCCTCAGGGACTTC 1050  
G S G N M E E E C E K F L R D F

1051 ACCGAGAACCCATGCCTCCGGAACGCCATCCAGGCCTTTGGCAACGGCAC 1100  
T E N P C L R N A I Q A F G N G T

1101 GGACGTGAACGTGTCCCCAAAAGGCCCTCGTTCCAGGCCACCCAGGCCC 1150  
D V N V S P K G P S F Q A T Q A P

1151 CTCGGGTGGAGAAGACGCCTTCTTTGCCAGATGACCTCAGTGACAGTACC 1200  
R V E K T P S L P D D L S D S T

1201 AGCTTGGGGACCAGTGTTCATCACCACCTGCACGTCTGTCCAGGAGCAGGG 1250  
S L G T S V I T T C T S V Q E Q G

1251 GCTGAAGGCCAACAACCTCCAAAGAGTTAAGCATGTGCTTCACAGAGCTCA 1300  
L K A N N S K E L S M C F T E L T

1301 CGACAAATATCATCCCAGGGAGTAACAAGGTGATCAAACCTAACTCAGGC 1350  
T N I I P G S N K V I K P N S G

1351 CCCAGCAGAGCCAGACCGTCCGGCTGCCTTGACCGTGCTGTCTGTCTGAT 1400  
P S R A R P S A A L T V L S V L M

1401 GCTGAAACTGGCCTTGTAGGCTGTGGGAACCGAGTCAGAAGATTTTTGAA 1450  
L K L A L

1451 AGCTACGCAGACAAGAACAGCCGCCTGACGAAATGGAAACACACACAGAC 1500

1501 ACACACACACCTTGCAAAAAAAAAAATTGTTTTTCCCACCTTGTCGCTGAA 1550

1551 CCTGTCTCCTCCCAGGTTTCTTCTCTGGAGAAGTTTTTGTAACCAAACA 1600

1601 GACAAGCAGGCAGGCAGCCTGAGAGCTGGCCCAGGGTCCCCTGGCAGGG 1650

1651 GAAACTCTGGTGCCGGGGAGGGCACGAGGCTCTAGAAATGCCCTTCACTT 1700

1701 TCTCCTGGTGTTTTTCTCTCTGGACCCTTCTGAAGCAGAGACCGGACAAG 1750

1751 AGCCTGCAGCGGAAGGGACTCTGGGCTGTGCCTGAGGCTGGCTGGGGGCA 1800

1801 GGACAACACAGCTGCTTCCCAGGCTGCCCACTCTGGGGACCCGCTGGGG 1850

1851 GCTGGCAGAGGGCATCGGTACGCGGGGCAGCGGGGCTG 1888

FIG. 7b

SUBSTITUTE SHEET (RULE 26)

1	MILANVFCFLFFFLDETLRSLASPSSSLQGPPELHGWRPPVDCVRANELCAAE	50
1	MFLATLYFALPLLDLLLSAEVSGGD.....RLDCVKASDQCLKE	39
51	SNCSSRYRTLROCLAGRDRN.....TMLANKECQAALEVLOESPLYDCRC	95
40	QSCSTKYRTLROQCVAGKETNFSLASGLEAKDECRSAMEALKQKSLYNCR	89
96	KRGMKKELOQCLQIYWSIHLGLTEGEEFYEASPYEPVTSRLSDIFRLASIF	145
90	KRGMKKEKNCLRIYWSMYQSL.QGNDLLEDSPYEPVNSRLSDIFRVVPFI	138
146	SGTGADPVVSAKSNHCLDAAKACNLNDNCKKLRSYISICNREISPTERC	195
139	SVEHI.....PKGNNCLDAAKACNLDDICKKYRSAYITPCTTSVS.NDVC	182
196	NRRKCHKALRQFFDRVPSEYTYRMLFCSCQDQACAERRRQTILPSCSYED	245
183	NRRKCHKALRQFFDKVPAKHSYGMLFCSCRDIACTERRRQTIVPVCSYEE	232
246	KEKPNCLDLRGVCRTDHLCSRSLADFHANCRASYQTVTSCPADNYQACLG	295
233	REKPNCLNLQDSCKTNYICRSLADFFTNCQPESRSVSSCLKENYADCLL	282
296	SYAGMIGFDMTPNYVDSSPTGIVVSPWCSCRGSGNMEEECEKFLRDFTEN	345
283	AYSGLIGTVMTPNYIDSS..SLSVAPWCDCSNSGNDLEECLKFLNFFKDN	330
346	PCLRNAIQAFNGGTDVNVSPKGPSFQATQAPRVEKTPSLPDDLSDSTS..	393
331	TCLKNAIQAFNGGSDVTWQPAFPVQTTTATTTTALRVKNKPLGPAGSEN	380
394	.LGTSVITTCTSVQEQGLKANNS..KELSMCFTELTTNIIPGSNKVIKPN	440
381	EIPTHVLPPCANLQAQKLKSNVSGNTHLCISNGNYEKEGLGASSHITTKS	430
441	SGPSRARPSAALTIVLSVLMKLAL	464
431	MAAPPSCGLSPLLVLVVTALSTLL	454

**SUBSTITUTE SHEET (RULE 26)**

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1 CGCAGGCAGAGCGCTGTCGCATCCCGGGCGTCCACCCGCCATGGGGCTCT 50  
M G L S

51 CCTGGAGCCCGCGACCTCCACTGCTGATGATCCTGCTACTGGTGCTGTCC 100  
W S P R P P L L M I L L L V L S

101 TTGTGGCTGCCACTTGGAGCAGGAAACTCCCTTGCCACAGAGAACAGGTT 150  
L W L P L G A G N S L A T E N R F

151 TGTGAACAGCTGTACCCAGGCCAGAAAGAAATGCGAGGCTAATCCCGCTT 200  
V N S C T Q A R K K C E A N P A C

201 GCAAGGCTGCCTACCAGCACCTGGGCTCCTGCACCTCCAGTTTAAGCAGG 250  
K A A Y Q H L G S C T S S L S R

251 CCGCTGCCCTTAGAGGAGTCTGCCATGTCTGCAGACTGCCTAGAGGCAGC 300  
P L P L E E S A M S A D C L E A A

301 AGAACAACTCAGGAACAGCTCTCTGATAGACTGCAGGTGCCATCGGCGCA 350  
E Q L R N S S L I D C R C H R R M

351 TGAAGCACCAAGCTACCTGTCTGGACATTTATTGGACCGTTACCCCTGCC 400  
K H Q A T C L D I Y W T V H P A

401 CGAAGCCTTGGTGACTACGAGTTGGATGTCTCACCTATGAAGACACAGT 450  
R S L G D Y E L D V S P Y E D T V

451 GACCAGCAAACCTGGAAAATGAATCTTAGCAAGTTGAACATGCTCAAAC 500  
T S K P W K M N L S K L N M L K P

501 CAGACTCGGACCTCTGCCTCAAATTTGCTATGCTGTGTA CTCTTACGAC 550  
D S D L C L K F A M L C T L H D

551 AAGTGTGACCGCCTGCGCAAGGCCTACGGGGAGGCATGCTCAGGGATCCG 600  
K C D R L R K A Y G E A C S G I R

601 CTGCCAGCGCCACCTCTGCCTAGCCCAGCTGCGCTCCTTCTTTGAGAAGG 650  
C Q R H L C L A Q L R S F F E K A

651 CAGCAGAGTCCCACGCTCAGGGTCTGCTGCTGTGTCCCTGTGCACCAGAA 700  
A E S H A Q G L L L C P C A P E

701 GATGCGGGCTGTGGGGAGCGGCGGCGTAACACCATCGCCCCAGTTGCGC 750  
D A G C G E R R R N T I A P S C A

751 CCTGCCTTCTGTAACCCCCAATTGCCTGGATCTGCGGAGCTTCTGCCGTG 800  
L P S V T P N C L D L R S F C R A

801 CGGACCCTTTGTGCAGATCACGCCTGATGGACTTCCAGACCCACTGTCAT 850  
D P L C R S R L M D F Q T H C H

851 CCTATGGACATCCTTGGGACTTGTGCAACTGAGCAGTCCAGATGTCTGCG 900  
P M D I L G T C A T E Q S R C L R

FIG. 9a

SUBSTITUTE SHEET (RULE 26)

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901 GGCATACCTGGGGCTGATTGGGACTGCCATGACCCCAAACCTTCATCAGCA 950  
A Y L G L I G T A M T P N F I S K

951 AGGTCAACACTACTGTTGCCTTAAGCTGCACCTGCCGAGGCAGCGGCAAC 1000  
V N T T V A L S C T C R G S G N

1001 CTACAGGACGAGTGTGAACAGCTGGAAAGGTCCTTCTCCCAGAACCCCTG 1050  
L Q D E C E Q L E R S F S Q N P C

1051 CCTCGTGGAGGCCATTGCAGCTAAGATGCGTTTCCACAGACAGCTCTTCT 1100  
L V E A I A A K M R F H R Q L F S

1101 CCCAGGACTGGGCAGACTCTACTTTTTTCAGTGGTGCAGCAGCAGAACAGC 1150  
Q D W A D S T F S V V Q Q Q N S

1151 AACCTGCTCTGAGACTGCAGCCCAGGCTACCCATTCTTTCTTTCTCCAT 1200  
N P A L R L Q P R L P I L S F S I

1201 CCTTCCCTTGATTCTGCTGCAGACCCTCTGGTAGCTGGGCTTCCTCAGG 1250  
L P L I L L Q T L W

1251 TCCTTTGTCCTCTCCACCACACCCAGACTGATTTGCAGCCTGTGGTGGGA 1300

1301 GAGAACTCGCCAGCCTGTGGAAGAAGACGCAGCGTGCTACACAGCAACCC 1350

1351 GGAACCAACCAGGCATTCCGCAGCACATCCCGTCTGCTCCAGAAGAGGTC 1400

1401 TTAGAAGTGAGGGCTGTGACCCTTCCGATCCTGAGCGGCTAGTTTTCAA 1450

1451 CCTCCCTTGCCCCTGCTTCCTTCTGGCTCAGGCTGCTCCTCCTTAGGACT 1500

1501 TTGTGGGTCCAGTTTTGCCTTCTGTTCTGATGGTGATTAGCGGCTCACCT 1550

1551 CCAGCGCTTCTTCCTGTTTCCCAGGACCACCCAGAGGCTAAGGAATCAGT 1600

1601 CATTCCCTGTTGCCTTCTCCAGGAAGGCAGGCTAAGGGTTCTGAGGTGAC 1650

1651 TGAGAAAAATGTTTCCTTTGTGTGGAAGGCTGGTGCTCCAGCCTCCACGT 1700

1701 CCCTCTGAATGGAAGATAAAAACCTGCTGGTGTCTTGACTGCTCTGCCAG 1750

1751 GCAATCCTGAACATTTGGGCATGAAGAGCTAAAGTCTTTGGGTCTTGTTT 1800

1801 AACTCCTATTACTGTCCCCAAATTCCCCTAGTCCCTTGGGTCATGATTAA 1850

1851 ACATTTTGACTTAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 1889

FIG. 9b

SUBSTITUTE SHEET (RULE 26)

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1 TGTGGACGCGCGCTTCGGAGTTGGAGGGCGGCGCCAGGACCCTGGTGGG 50  
51 AGAGTGTGTGCGTCGCGCTGGAGGGCGGGAGGCGGGGCGGGAGGTGCCG 100  
101 GTCGAGGGAGCCCCGCTCTCAGAGCTCCAGGGGAGGAGCGAGGGGAGCGC 150  
151 GGAGCCCGGCGCCTACAGCTCGCCATGGTGCGCCCCCTGAACCCGCGACC 200  
M V R P L N P R P  
201 GCTGCCGCGCGTAGTCCTGATGTTGCTGCTGCTGCTGCCGCGCTCGCCG 250  
L P P V V L M L L L L P P S P L  
251 TGCCTCTCGCAGCCGGAGACCCCTTCCCACAGAAAGCCGACTCATGAAC 300  
P L A A G D P L P T E S R L M N  
301 AGCTGTCTCCAGGCCAGGAGGAAGTGCCAGGCTGATCCCACCTGCAGTGC 350  
S C L Q A R R K C Q A D P T C S A  
351 TGCCTACCACCACCTGGATTCTGACCTCTAGCATAAGCACCCCACTGC 400  
A Y H H L D S C T S S I S T P L P  
401 CCTCAGAGGAGCCTTCGGTCCCTGCTGACTGCCTGGAGGCAGCACAGCAA 450  
S E E P S V P A D C L E A A Q Q  
451 CTCAGGAACAGCTCTCTGATAGGCTGCATGTGCCACCGGCGCATGAAGAA 500  
L R N S S L I G C M C H R R M K N  
501 CCAGGTTGCCTGCTTGGACATCTATTGGACCGTTCACCGTGCCCGCAGCC 550  
Q V A C L D I Y W T V H R A R S L  
551 TTGGTAACTATGAGCTGGATGTCTCCCCCTATGAAGACACAGTGACCAGC 600  
G N Y E L D V S P Y E D T V T S  
601 AAACCCTGGAAAATGAATCTCAGCAAACCTGAACATGCTCAAACCAGACTC 650  
K P W K M N L S K L N M L K P D S  
651 AGACCTCTGCCTCAAGTTTGCCATGCTGTGTACTCTCAATGACAAGTGTG 700  
D L C L K F A M L C T L N D K C D  
701 ACCGGCTGCGCAAGGCCTACGGGGAGGCGTGCTCCGGGCCCCACTGCCAG 750  
R L R K A Y G E A C S G P H C Q  
751 CGCCACGTCTGCCTCAGGCAGCTGCTCACTTTCTTCGAGAAGGCCGCGGA 800  
R H V C L R Q L L T F F E K A A E  
801 GCCCCACGCGCAGGGCCTGCTACTGTGCCCATGTGCCCCCAACGACCGGG 850  
P H A Q G L L L C P C A P N D R G  
851 GCTGCGGGGAGCGCCGGCGCAACACCATCGCCCCCAACTGCGCGCTGCCG 900  
C G E R R R N T I A P N C A L P  
901 CCTGTGGCCCCCAACTGCCTGGAGCTGCGGCGCCTCTGCTTCTCCGACCC 950  
P V A P N C L E L R R L C F S D P

FIG. 10a

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20/20

951 GCTTTGCAGATCACGCCTGGTGGATTTCCAGACCCACTGCCATCCCATGG 1000  
L C R S R L V D F Q T H C H P M D

1001 ACATCCTAGGAACTTGTGCAACAGAGCAGTCCAGATGTCTACGAGCATAC 1050  
I L G T C A T E Q S R C L R A Y

1051 CTGGGGCTGATTGGGACTGCCATGACCCCCAACTTTGTGCAATGTCAA 1100  
L G L I G T A M T P N F V S N V N

1101 CACCAGTGTTGCCTTAAGCTGCACCTGCCGAGGCAGTGGCAACCTGCAGG 1150  
T S V A L S C T C R G S G N L Q E

1151 AGGAGTGTGAAATGCTGGAAGGGTTCTTCTCCCACAACCCCTGCCTCAGG 1200  
E C E M L E G F F S H N P C L T

1201 GAGGCCATTGCAGCTAAGATGCGTTTTTCACAGCCAACTCTTCTCCCAGGA 1250  
E A I A A K M R F H S Q L F S Q D

1251 CTGGCCACACCCTACCTTTGCTGTGATGGCACACCAGAATGAAAACCCTG 1300  
W P H P T F A V M A H Q N E N P A

1301 CTGTGAGGCCACAGCCCTGGGTGCCCTCTCTTTTCTCCTGCACGCTTCCC 1350  
V R P Q P W V P S L F S C T L P

1351 TTGATTCTGCTCCTGAGCCTATGGTAGCTGGACTTCCCCAGGGCCCTCTT 1400  
L I L L L S L W

1401 CCCCTCCACCACACCCAGGTGGACTTGCAGCCCACAAGGGGTGAGGAAAG 1450

1451 GACAGCAGCAGGAAGGAGGTGCAGTGCGCAGATGAGGGCAGGAGAAGC 1500

1501 TAAGGGTTATGACCTCCAGATCCTTACTGGTCCAGTCCTCATTCCTCCA 1550

1551 CCCCATCTCCACTTCTGATTCATGCTGCCCCTCCTTGGTGGCCACAATTT 1600

1601 AGCCATGTCATCTGGTGCCTGTGGGCCTTGCTTTATTCTATTATTGTCC 1650

1651 TAAAGTCTCTCTGGGCTCTTGGATCATGATTAAACCTTTGACTTAAAAA 1699

FIG. 10b

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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C07K 14/47, C12N 15/12, C12Q 1/68,</b> <b>A61K 48/00, C12N 5/10, 15/62, C07K</b> <b>16/18, C12N 5/06, G01N 33/566</b>	<b>A3</b>	<b>(11) International Publication Number:</b> <b>WO 97/44356</b>  <b>(43) International Publication Date:</b> 27 November 1997 (27.11.97)
<b>(21) International Application Number:</b> PCT/US97/07726  <b>(22) International Filing Date:</b> 7 May 1997 (07.05.97)  <b>(30) Priority Data:</b> 60/017,427 8 May 1996 (08.05.96) US 60/019,300 7 June 1996 (07.06.96) US 60/021,859 16 July 1996 (16.07.96) US Not furnished 10 April 1997 (10.04.97) US  <b>(60) Parent Application or Grant</b> <b>(63) Related by Continuation</b> US 60/017,427 (CIP) Filed on 8 May 1996 (08.05.96)  <b>(71) Applicant (for all designated States except US):</b> BIOGEN, INC. [US/US]; 14 Cambridge Center, Cambridge, MA 02142 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> SANICOLA-NADEL, Michele [US/US]; 4 Maple Road, Winchester, MA 01890 (US). HESSION, Catherine [US/US]; 35 Otis Hill Road,		Hingham, MA 02043 (US). CATE, Richard, L. [US/US]; 64 Arrowhead Road, Weston, MA 02193 (US).  <b>(74) Agent:</b> LEVINE, Leslie, M.; Biogen, Inc., 14 Cambridge Center, Cambridge, MA 02142 (US).  <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>  <b>(88) Date of publication of the international search report:</b> 19 February 1998 (19.02.98)
<b>(54) Title:</b> RET LIGAND (RetL) FOR STIMULATING NEURAL AND RENAL GROWTH  <b>(57) Abstract</b>  This invention relates to nucleotide sequences which encode a Ret ligand (RetL), as well as to methods of stimulating neural and renal growth by treating cells and mammalian subjects with RetL DNA or protein. The invention provides a purified and isolated DNA molecule coding for a RetL, having the nucleotide sequence of any RetL, but specifically including rat retL 1 cDNA (SEQ ID NO:1), partial human RetL1 cDNA (SEQ ID NO:8), full length human retL 1 cDNA (SEQ ID NO:10), human retL2 cDNA (SEQ ID NO:12), murine retL3 cDNA (SEQ ID NO:16), partial human retL3 cDNA (SEQ ID NO:18) or human retL3 cDNA (SEQ ID NO:20). The invention further provides a RetL protein, with an amino acid sequence comprising that of rat RetL1 (SEQ ID NO:2), partial human RetL1 (SEQ ID NO:9), full length human RetL (SEQ ID NO:11), human RetL2 (SEQ ID NO:13), murine RetL3 (SEQ ID NO:17), partial human RetL3 (SEQ ID NO:19) or human RetL3 (SEQ ID NO:21).		

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# INTERNATIONAL SEARCH REPORT

Intern. al Application No

PCT/US 97/07726

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07K14/47 C12N15/12 C12Q1/68 A61K48/00 C12N5/10  
C12N15/62 C07K16/18 C12N5/06 G01N33/566

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K C12Q A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HILLIER, L. ET AL.: "The WashU-Merck EST Project - y170a10.s1 Homo sapiens cDNA clone 43207 3' " EMBL DATABASE ENTRY HS619153; ACCESSION NUMBER H05619, 23 June 1995, XP002047612 see abstract	1,4
X	HILLIER, L. ET AL.: "The WashU-Merck EST Project - ye83h05.s1 Homo sapiens cDNA clone 124377 3' " EMBL DATABASE ENTRY HS13571; ACCESSION NUMBER R02135 , 17 April 1995, XP002047613 see abstract	1,4

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

20 November 1997

Date of mailing of the international search report

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## INTERNATIONAL SEARCH REPORT

Intern Application No

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PANDEY A ET AL: "The Ret receptor protein tyrosine kinase associates with the SH2-containing adapter protein Grb10." JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 SEP 15) 270 (37) 21461-3., XP002047614 see abstract ---	31,32, 39-42
A	CAO T.: "c-ret and signal transduction." CANCER BULLETIN, (1995) 47/2 (119-124)., XP002047615 ---	
A	PASINI B ET AL: "RET mutations in human disease" TRENDS IN GENETICS, vol. 12, no. 4, April 1996, page 138-144 XP004037254 cited in the application ---	
A	WO 95 16709 A (BIOGEN INC ;INSERM U 293 (FR); CATE RICHARD L (US); JOSSO NATHALIE) 22 June 1995 cited in the application see page 2, line 32 - page 4, line 3 see page 15, line 19 - page 18 see page 22, line 11 - page 24, line 23 ---	14-19, 22-42
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P,X	HILLIER, ET AL.: "The WashU-Merck EST Project - mj1ld08.r1 Soares mouse embryo MbME13.5 14.5 Mus musculus cDNA clone 475791 5' " EMBL DATABASE ENTRY MMAA49894; ACCESSION NUMBER AA049894 (VERSION 2), 31 December 1996, XP002047617 cited in the application see abstract ---	1,4
P,A	WO 97 18240 A (IBANEZ CARLOS ;ARENAS ERNEST (SE); ARUMAE URMAS (FI); FAINZILBER M) ---	1-47
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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 97/07726

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 97 33912 A (GENENTECH INC ;KLEIN ROBERT D (US); MOORE MARK W (US); ROSENTHAL A) see the whole document ---	1-47
T	MASON, I.: "The GDNF receptor: Recent progress and unanswered question" MOL. CELL. NEUROSCI., (1996) VOL. 8, NO. 2-3, PP. 112-119., XP002047618 ---	
T	ROBERTSON K ET AL: "The GDNF-RET signalling partnership" TRENDS IN GENETICS, vol. 13, no. 1, January 1997, page 1-3 XP004015047 -----	

# INTERNATIONAL SEARCH REPORT

Int'l application No.  
PCT/US 97/07726

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
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4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
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# INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 97/07726

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claims 30-34,36,37,39-41 (as far as in vivo methods are concerned) and claims 22-29,35,38,42-47 are directed methods of treatment or diagnostic of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 97/07726

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-30, 33-38, 43-47 (all partially)

A ret ligand, RetL1, nucleotide sequences coding therefore, vectors and host cells containing them, variants therefrom, antibodies against it, and its uses.

2. Claims: 1-30, 33-38, 43-47 (all partially)

As for subject 1, but concerning the ret ligand RetL2.

3. Claims: 1-30, 33-38, 43-47 (all partially)

As for subject 1, but concerning the ret ligand RetL3.

4. Claims: 31-32, 39-42

Methods using variants of the Ret protein.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern

ial Application No

PCT/US 97/07726

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9516709 A	22-06-95	AU 1404395 A	03-07-95
WO 9718240 A	22-05-97	AU 1158897 A	05-06-97
WO 9733912 A	18-09-97	NONE	

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